STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL. XVII. THE INFLUENCE OF AGE UPON REPRODUCTIVE ABILITY, WITH A DESCRIPTION OF A NEW REPRODUCTIVE INDEX[1]

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THE PROBLEM

There is a widespread opinion among poultry breeders, which has found its way into the body of poultry husbandry teaching, that it is desirable to use birds two years or more of age for breeding purposes rather than pullets and cockerels one year of age at the time they are bred. Various reasons have been alleged for this view, such as that the progeny from older birds is superior in vigor, or that a more numerous progeny is had per mating, etc. There has never been presented, however, so far as I am aware, any definite quantitative evidence in adequate amount from which one could derive a just conclusion about the influence of age upon the breeding ability of the domestic fowl.

The matter is one of considerable theoretical interest as well as practical importance. With the modern intensive study of genetics the relation of all matters having to do with the physiology of breeding to the age of the animals bred takes on increased theoretical importance. On the practical side it is highly desirable that the breeder should know whether he is actually or potentially reducing the efficiency of his breeding operations if he uses pullets and cockerels as breeders.

It is the purpose of this paper to present some exact numerical evidence regarding the influence of age upon reproductive capacity in fowls of the Barred Plymouth Rock breed. The data on which the paper is based represent the writer's experience at the MAINE AGRICULTURAL EXPERIMENT STATION with this breed for a period of nine consecutive years, during which time exact records have been kept of all the chief factors involved in the reproduction. The number of separate individual

[1] Papers from the Biological Laboratory of the MAINE AGRICULTURAL EXPERIMENT STATION, No. 110.

matings involved is large and it is believed that the results probably represent with substantial accuracy the conditions which will be found to hold generally for this breed.

In attempting the solution of the problem of the influence of age upon reproductive capacity the first essential is an adequate measure of this character. If one considers carefully the question of the measurement of total reproductive capacity in poultry it is at once evident that there are several different factors involved. First and most fundamental is obviously fecundity.[2] The size of the family which any given mating leaves will evidently in the first instance be determined by the number of eggs which the hen taking part in the mating lays during the breeding period. If she produces only a few eggs, of necessity there can be but few offspring. If she lays many eggs, there may be many or few offspring, the results then being determined by other factors.

The second factor involved in reproductive capacity will be the fertilization of the eggs. If for any reason the male bird in the mating fails to fertilize the eggs, or if the chemical conditions in the oviduct of the female are such as to make it impossible for fertilization to take place, then the number of progeny will be small even though many eggs are laid. The percentage of infertile eggs to the total number of eggs laid is obviously an important factor in the total reproductive capacity of the fowl.

The third important factor is prenatal mortality. A hen may lay many eggs and these may show a high percentage of fertility, but if the embryos are so weak and lacking in vitality that they die before the end of incubation the actual progeny left by the pair of birds in the mating will be by so much reduced. It has been shown by PEARL and

[2] It is desirable to repeat here the working definition of the terms fecundity and fertility which the writer proposed some years ago, and which have been used throughout the work in this laboratory on problems relating to reproduction in the domestic fowl. PEARL and SURFACE (1909 a) defined these terms as follows: "We would suggest that the term 'fecundity' be used only to designate the innate potential reproductive capacity of the individual organism, as denoted by its ability to form and separate from the body mature germ cells. Fecundity in the female will depend upon the production of ova and in the male upon the production of spermatozoa. In mammals it will obviously be very difficult, if not impossible, to get reliable quantitative data regarding pure fecundity. On the other hand we would suggest that the term 'fertility' be used to designate the total actual reproductive capacity of pairs of organisms, male and female, as expressed by their ability when mated together to produce (i. e., bring to birth) individual offspring. Fertility, according to this view, depends upon and includes fecundity, but also a great many other factors in addition. Clearly it is fertility rather than fecundity which is measured in statistics of birth of mammals."

SURFACE (1909 b) that these two factors, percentage of infertile eggs and percentage of prenatal mortality, are rather highly variable, and to a considerable extent independently of each other. That is, high prenatal mortality may be associated with either high or low fertility of eggs.

The next important factor in the actual reproductive capacity of a bird is the early postnatal mortality. From a broad biological viewpoint the effectiveness of the reproductive processes is measured by the preservation of the species. Any pairing of individuals must leave behind itself sufficient progeny to ensure that some come to maturity and in turn form a new generation. Any of the progeny individuals that die before reaching sexual maturity obviously represent by so much a defective element in the reproductive process. It is a fact well known to poultry breeders that the major portion of chick mortality takes place within the first three weeks after hatching. Under exceptional circumstances, as of epidemics of some contagious disease, there may be a heavy mortality after the birds have attained three weeks of age. The deaths due to innate weakness and lack of constitutional vigor, however, in the main occur, as experience shows, within the first three weeks after hatching. A very large percentage of the chicks alive at three weeks of age may be expected, barring accidents, to live to sexual maturity.

Any quantitative measure of reproductive capacity in the domestic fowl must take into account all of these factors which have been named. They are all variable in rather high degree, and while there is a sensible correlation between some of them in their variation, these correlations are in general low. To put the matter in the other way, these various factors on which reproductive capacity in the domestic fowl primarily depends are, to a considerable degree, independently variable.

Some years ago a poultry breeding index, or, as it was then called a "selection index," was proposed by PEARL and SURFACE (1909 c). The index then proposed was described as follows:

$$I_1 = \frac{5(a+b)}{c+d+1}$$

The following scheme shows the meaning of the letters in the formula:

I_1 = general or fundamental poultry selection index for an individual bird.

a = percentage of this bird's eggs which hatched.

b = percentage of eggs actually laid by this bird to the total number it was possible for her to lay between February 1 and

June 1 (i. e., the breeding season) of the year for which the index is calculated.

c = percentage of this bird's eggs which were infertile.

d = percentage of chicks hatched from this bird's eggs which died within three weeks from the date of hatching.

Practical experience in the use of this index has shown that it has certain defects and fails on that account to meet fully the needs of a satisfactory measure of the total reproductive performance of a mating. A good deal of study has been devoted to the matter of determining a thoroughly satisfactory measure. In the first place, it early became clear that it was a mistake to consider the matter primarily with reference to the female partner in the mating alone. Reproductive capacity or performance in sexually reproducing organisms is obviously a property or characteristic of *matings;* not of individuals. In the second place, it became evident that the more or less independent character of the variation in the several variables involved made the very crude scheme of combination adopted in the original index altogether inadequate. A great deal of time was spent in trying to devise a more complicated and at the same time more adequate scheme of combination of the several variables. While some success was attained, the results were always far from what could be desired.

A NEW REPRODUCTIVE INDEX FOR POULTRY

After much work on the subject it was finally decided to approach the whole problem of the measurement of the total reproductive capacity from a new point of view. This led to the adoption of a new reproductive index for poultry which may be described in the following way:

$$RI = \frac{\textit{Number of chickens alive at the end of the 3rd week after hatching} \times 100}{\textit{Total number of days from the day when this mating began to the day when the last egg from this mating began its incubation}}$$

The reasoning on which this index is based is as follows: Maximum reproductive capacity, as represented by 100 percent, would be attained if, during the period of the mating, the hens laid one egg every day (maximum fecundity), and if further every one of such eggs was fertile, and if each embryo hatched, and the hatched chick lived to be three weeks of age. There would then be one living chick three weeks of age for each day during which the mating existed. If the hen does *not* lay every day during the mating season this will cause some reduction in the reproductive performance as measured by the index. Similarly a reduction in any of the other factors involved, prenatal or postnatal mortality, will have the same sort of result. The final percentage value which one

obtains by calculating the index will be a true measure of the reproductive capacity of that mating, including within its view *all* of the primary factors of reproduction in poultry.

This proposed index is obviously much simpler to calculate than the old one, and its very simplicity, directness, and obviously logical character commend it at once as the measure for which we are seeking. Actual experience with the index in dealing with data from our matings over a series of years shows that it is an extremely satisfactory and reliable measure of total reproductive performance. It gives a unique numerical expression of just the thing one wants to know, namely the degree to which a mating or pairing of individuals effectively perpetuated itself.

THE INFLUENCE OF AGE UPON REPRODUCTIVE PERFORMANCE IN THE FOWL

We may now turn to a consideration of the problem with which this paper has primarily to do, using the reproductive index as the instrument of research. During the past year my assistant, Mr. JOHN RICE MINER, has calculated the value of this index for all matings which have been made at the MAINE AGRICULTURAL EXPERIMENT STATION poultry plant during the last nine years. In the present paper we shall deal only with the Barred Plymouth Rock matings. Table 1 shows the

TABLE 1

Frequency distribution of reproductive index for Barred Plymouth Rock matings.

Reproductive index	Frequency	Reproductive index	Frequency
- 1.4	195	31.5-33.4	20
1.5- 3.4	68	33.5-35.4	11
3.5- 5.4	88	35.5-37.4	11
5.5- 7.4	82	37.5-39.4	9
7.5- 9.4	83	39.5-41.4	4
9.5-11.4	69	41.5-43.4	4
11.5-13.4	75	43.5-45.4	4
13.5-15.4	83	45.5-47.4	1
15.5-17.4	64	47.5-49.4	1
17.5-19.4	52	49.5-51.4	0
19.5-21.4	47	51.5-53.4	0
21.5-23.4	34	53.5-55.4	0
23.5-25.4	35	55.5-57.4	0
25.5-27.4	33	57.5-59.4	1
27.5-29.4	23	59.5-61.4	2
29.5-31.4	15	Total	1114

Mean	12.496 ± 0.212
Standard deviation	10.495 ± 0.150
Coefficient of variation	83.988 ± 1.864

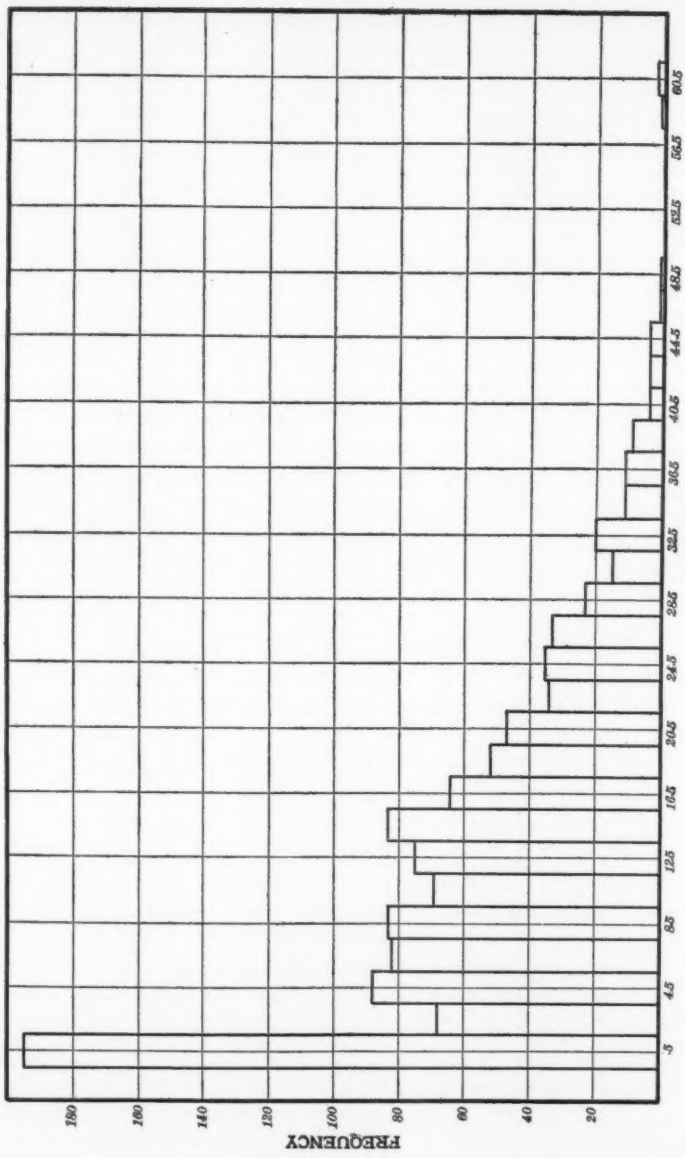

FIGURE 1.—Histogram showing variation in reproductive index.

TABLE 2

Showing the distribution of the reproductive index for each age of sire and dam.

Reproductive index	Age of Sire=1 Dam=1	Sire=1 Dam=2	Sire=1 Dam=3	Sire=1 Dam=4	Sire=1 Dam=5	Sire=2 Dam=1	Sire=2 Dam=2	Sire=2 Dam=3	Sire=3 Dam=1	Sire=3 Dam=2	Sire=3 Dam=3	Sire=4 Dam=2
—1.4	136	23	1	—	—	12	13	1	6	3	—	—
1.5—3.4	44	10	1	1	—	1	6	1	4	0	—	—
3.5—5.4	57	19	0	—	1	3	6	1	1	0	—	—
5.5—7.4	61	10	0	—	—	3	5	—	2	1	—	—
7.5—9.4	63	11	0	—	—	3	4	—	2	0	—	—
9.5—11.4	46	17	0	—	—	2	1	—	2	1	—	—
11.5—13.4	49	8	2	—	—	4	9	—	2	0	1	—
13.5—15.4	51	13	0	—	—	2	12	—	3	2	—	—
15.5—17.4	51	4	0	—	—	2	7	—	0	0	—	—
17.5—19.4	38	8	1	—	—	3	1	—	1	0	—	—
19.5—21.4	32	6	0	—	—	1	7	—	1	0	—	—
21.5—23.4	21	7	0	—	—	0	4	—	2	0	—	—
23.5—25.4	32	2	0	—	—	0	1	—	0	0	—	—
25.5—27.4	27	4	1	—	—	0	1	—	0	0	—	—
27.5—29.4	21	1	0	—	—	0	1	—	0	0	—	—
29.5—31.4	14	0	0	—	—	0	0	—	0	0	—	1
31.5—33.4	15	2	1	—	—	0	0	—	0	0	—	—
33.5—35.4	10	0	—	—	—	1	0	—	1	1	—	—
35.5—37.4	8	3	—	—	—	—	0	—	—	—	—	—
37.5—39.4	5	3	—	—	—	—	1	—	—	—	—	—
39.5—41.4	4	0	—	—	—	—	—	—	—	—	—	—
41.5—43.4	3	1	—	—	—	—	—	—	—	—	—	—
43.5—45.4	3	1	—	—	—	—	—	—	—	—	—	—
45.5—47.4	1		—	—	—	—	—	—	—	—	—	—
47.5—49.4	1	—	—	—	—	—	—	—	—	—	—	—
49.5—51.4	0	—	—	—	—	—	—	—	—	—	—	—
51.5—53.4	0	—	—	—	—	—	—	—	—	—	—	—
53.5—55.4	0	—		—	—	—	—	—	—	—	—	—
55.5—57.4	0	—	—	—	—	—	—	—	—	—	—	—
57.5—59.4	1	—	—	—	—	—	—	—	—	—	—	—
59.5—61.4	2	—	—	—	—	—	—	—	—	—	—	—
Totals	796	153	7	1	1	37	79	3	27	8	1	1

value of the reproductive index for 1114 matings of pure-bred Barred Plymouth Rocks, all ages being included in this distribution which is shown graphically in figure 1. A wide range of environmental conditions is represented in the statistics. Some of the years contributing data were extremely good years for hatching and rearing, while others were just as extremely bad. On the whole the statistics are so extensive and the time covered so long that the end result probably is about the average representation of the breed.

From the table and diagram we note at once the following points:

1. There is an unexpectedly large amount of variation in this characteristic. That is, the values obtained from particular matings range all the way from 0 to over 60 percent.

2. Leaving out of account for a moment the first class (values of the index from 0 to 1.5) we see a rather flat-topped distribution over to a value of about 18 percent for the index. Beginning at that point and continuing to the end of the distribution there is a steady falling off in the frequency with high indices.

3. The high value of the first class, including reproductive indices from 0 to 2, arises from the considerable number of wholly sterile matings which give a value for the reproductive index of 0.

In table 2 the data are so arranged as to show the relation of reproductive capacity to age. Separate distributions are given for each age grouping of sire and dam.

The variation constants, with their probable errors, deduced from table 2 are shown in table 3. SHEPPARD's correction of the second moment was not used, the conditions which warrant its use not being even approximately realized in the distributions.

TABLE 3

Showing the variation constants for reproductive index in Barred Plymouth Rocks.

Age of sire and dam	Mean	Standard deviation	Coefficient of variation
Sire=1, Dam=1	13.083±0.260	10.867±0.184	83.066± 2.166
Sire=1, Dam=2	11.781±0.546	10.016±0.386	85.018± 5.127
Sire=1, Dam=3	15.071±2.775	10.887±1.963	72.237±18.615
Sire=1, Dam=4	2	0	0
Sire=1, Dam=5	4	0	0
Sire=2, Dam=1	8.392±0.866	7.808±0.612	93.039±12.057
Sire=2, Dam=2	11.361±0.623	8.204±0.440	72.216± 5.539
Sire=2, Dam=3	2.500±0.636	1.633±0.450	65.320±24.486
Sire=3, Dam=1	9.389±1.086	8.368±0.768	89.127±13.163
Sire=3, Dam=2	10.000±2.429	10.186±1.718	101.858±30.118
Sire=3, Dam=3	13	0	0
Sire=4, Dam=2	30	0	0

From this table we note:

1. In round figures the reproductive index, in the case of the strain of Barred Rocks here dealt with, and under the general conditions of environment and management at the MAINE STATION poultry plant (PEARL 1916), has an average or mean value of 12.5. It is at present impossible to make any comparisons of these reproductive index figures for the reason that these constants have not been calculated for other conditions. From his own unpublished data, however, the writer is very sure that there will be found large reproductive index differences between different breeds. It is also highly probable that this constant will be found to take different values as climatic and other general environmental factors change.

2. The variation among different matings in respect of reproductive index is relatively large, as shown by the standard deviations and coefficients of variation. It is similar in this respect to variation in other physiological, as contrasted with structural, characters (PEARL 1905). In the present case this high variability is certainly only what would be expected, since the value taken by the reproductive index depends on the interaction of a series of physiological factors or processes, each of which is rather highly variable taken by itself.

3. Turning to the question of the effect of age upon the reproductive index we see that, having due regard to probable errors and the number of matings involved, *the highest mean value of the index in the case of these Barred Plymouth Rock matings is found when sire and dam are each one year old at the time of the mating.* The only higher *mean* in the table is that for sire = 1, dam = 3, and in that case only 7 matings are available, a number far too small to base a result upon.

The fact that the net reproductive results with cockerels and pullets are superior to those with older birds is directly contrary to the teaching of most of the poultry authorities. It is usually taught that the eggs of pullets are very apt to be infertile, and that the chicks which are obtained will be greatly lacking in vitality. The only recent writer I have found who does not subscribe strongly to such views is ROBINSON (1912), and even he inclines a little to the notion that, even though cockerel—pullet matings do produce a more numerous progeny than matings of older birds, the offspring of the younger birds may lack in "quality." "Quality" is an elusive term, not capable of numerical, quantitative statement. What the present statistical data show is that on the basis of the chick's *ability to live* as an index of constitutional vigor and vitality (and the writer's ten years experience with poultry has

shown him no better index of these qualities than this same ability to live) the net reproductive results are on the average *superior,* in the case of the MAINE STATION Barred Rock stock, when the parents are from 10 to 14 months of age at the time when the matings are made than if they are older. Whether or not this same relation holds for other breeds, or strains, and methods of management is a matter of great practical as well as theoretical interest. At present the writer knows of no reliable quantitative data sufficient in amount to base an opinion upon regarding this point.

4. Upon the question of the relative changes of the two sexes in reproductive ability, with advancing age, the following weighted mean figures for *males* may first be examined. The weighting in each case is in proportion to the number of matings involved, as indicated in table 2.

Weighted mean reproductive indices for males of specified ages mated with females of all ages.

Ages	Weighted mean *RI*
Male = 1, mated with ♀♀ of all ages	12.868
Male = 2, " " " " " "	10.214
Male = 3, " " " " " "	9.625

Similar figures for *females* may next be examined.

Weighted mean reproductive indices for females of specified ages mated with males of all ages.

Ages	Weighted mean *RI*
Female = 1, mated with ♂♂ of all ages	12.765
Female = 2, " " " " " "	11.660
Female = 3, " " " " " "	11.455

These data are shown graphically in fig. 2.

From these figures it appears clearly that there is a decline in net reproductive ability, as measured by the reproductive index, with advancing age in both sexes. The rate of the decline is, however, more rapid in the male than in the female. This result is what might reasonably be expected in a polygamous animal such as the fowl. A single male mates with a number of females, and in addition is much more active, on the whole, than the females, what with fighting, looking after his flock, etc. The writer judges it to be the experience of poultrymen generally that it is much more difficult to keep a cock bird in good condition for breeding at an advanced age than it is so to keep hens.

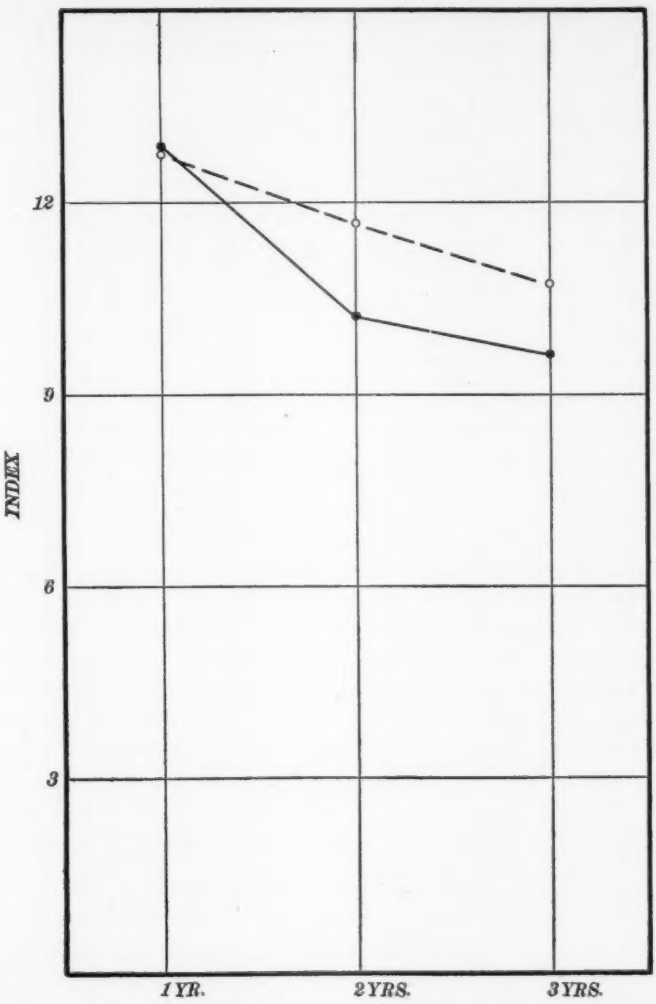

FIGURE 2.—Showing the change in reproductive index with advancing age, for males and females. Solid line and circles = males; broken line and open circles = females.

5. In the preceding section we have considered the change in reproductive ability with age in the sexes separately. It is desirable also to look at the matter from the standpoint of the *mating*. This may be done by taking means (weighted in proportion to the frequencies in-

volved) of the reproductive indices for the advancing ages of the two animals entering into each class of matings as given in tables 2 and 3. If this is done we get the following results.

Weighted mean reproductive indices for matings of individuals of the specified combined ages.

Combined ages of mated individuals when mated	Cases	Weighted mean *RI*
2 years	796	13.083
3 years	190	11.121
4 years	113	11.119
5 years	12	7.458
6 years	3	15.667

The cases are too few to give reliable results after a combined age of 4 years. Up to that point, however, what occurs is this: there is a significant drop in reproductive ability as we pass from a combined age of 2 years for the mated birds to 3 years. In passing from 3 years to 4 years there is no significant change in reproductive ability. In passing from a combined age of 4 years to that of 5 years there is a large drop in the net reproductive ability of the mating. The number of matings where the combined age was 5 years is, to be sure, small and too great dependence is not to be put upon the mean. It is however highly probable that there is some drop in reproductive ability at this point, though it may not be so great in amount as is indicated by the present figures. The number of cases of matings with a 6 year combined age is altogether too small to have any meaning whatever. Taken as a whole the present figures bear out very well the conclusion already reached that, for the strain of birds and the environmental conditions here dealt with, the highest net reproductive ability is found in birds one year old (cockerels and pullets) at the time they are mated.

The combined age means are shown graphically in fig. 3.

DISCUSSION

The most significant general result of the present study is the new index proposed for the measurement of the net reproductive ability of matings of the fowl. An adequate measure of this character has been much needed, and opens out the way to the attack on a number of problems. Such questions as the relation of reproductive ability to breed and variety, to the long-continued administration of drugs such as alcohol, ether, etc., to inbreeding, and the degree of heterosis, and many

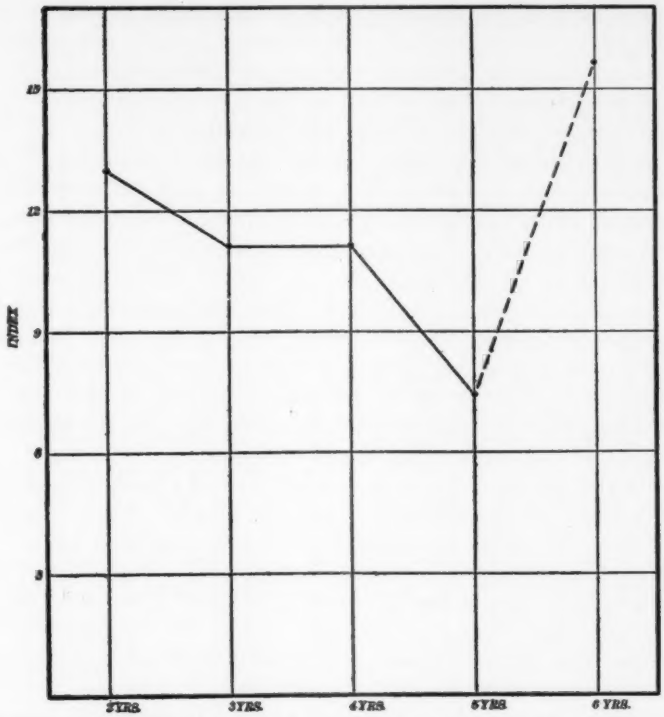

FIGURE 3.—Showing the change in reproductive index with advancing combined ages of the mated individuals. The dotted line signifies that in this region of the graph the data are too few to give reliable results.

other similar problems may now be profitably attacked. The writer hopes to return to the discussion of some of these problems in later papers. It is important that one caution be observed in applying this new reproductive index in the treatment of general problems. It is necessary that the duration of all matings compared in respect of reproductive index be substantially the same. The reason for this is that the value of the index will be somewhat affected by the length of time covered by the mating. Of course, in ordinary breeding practice this condition will be realized usually, but it will be well to keep the point in mind in making use of the index.

The character which has been called "reproductive ability" is practically the same thing as "fertility" as defined by PEARL and SURFACE

(1909 a). The only essential difference is that here the ability of the offspring to live is taken into account, whereas in the earlier definition fertility was taken to mean only bringing to birth. On the whole it seems now that the underlying idea of the present paper gives a more adequate and useful conception of fertility than the earlier one. We might then define fertility as the total net reproductive capacity of pairs of organisms, male and female, as indicated by their ability to produce *viable* individual offspring. As a working measure of fertility may be taken a reproductive or fertility index which expresses the percentage which the number of viable offspring actually produced from a particular mating or pair of parents is of the maximum number which would be physiologically possible within the time limits during whch the mating endures. This states in most general terms the form of index developed in this paper for the special case of poultry breeding. The same idea can be adapted to the measurement and biometrical study of fertility in other sorts of animals, and probably in plants as well.

Turning to the special problem with which this paper deals, the influence of age upon the fertility of matings of the fowl, we find that for the strain of Barred Plymouth Rocks and the environmental conditions here dealt with, maximum reproductive capacity is, on the average, attained in both sexes in the first breeding season. Never after that is the average fertility (as above defined) so high. It is of course to be understood that the pullets and cockerels involved in these statistics, and on the basis of which the above statement is made, were well matured birds, in no case less than 10 months old when mated, and from that to 14 months. Sexual maturity, as evidenced by the physiological ability to produce offspring, is regularly attained at an earlier age than this, and occasionally at a very much earlier one. The writer has in his possession now a brood of chicks, the *combined* ages of whose parents amounted to only one year. They are small and inferior birds. It is undoubtedly in considerable part as a result of breeding from such very young birds that the prejudice against the use of pullets and cockerels as breeders at all has arisen among poultrymen, and led to such statements in the texts as the following from LEWIS (1913):

> "When pullets are used as breeders, a large percentage of the eggs set are infertile, undoubtedly as a result of immaturity. The chicks at hatching time and maturity prove to be small."

There are undoubtedly a number of factors concerned in the decline of net reproductive capacity after birds have passed the first breeding

season. One important one is that emphasized by ROBINSON (1912) who says:

"It is largely a question of condition. The older a bird grows, the more difficult it is to keep it in good breeding condition. Few fowls and ducks are as good breeders the third year as the second, fewer still are good after the third year; yet occasionally four- and five-year-old birds of both sexes will breed as well and the hens lay as well as young stock, and there are authentic instances of fowls breeding well at seven and eight years of age."

MARSHALL (1908) has shown that general environmental conditions, nutrition, etc., may affect fertility in mammals. Besides these external factors in the decline there are also, without question, important internal factors operating in the same direction. VON HANSEMANN (1913) and KÄPPELI (1908) have shown that there is a progressive diminution of oöcytes in the ovary with advancing age. The analysis of the relative importance of these various factors in changing fertility is a problem of the future. It is believed that the possession of a simple and adequate measure of net fertility will aid materially in the study of this problem.

SUMMARY

1. A new constant, the reproductive or fertility index, is proposed for the measurement of the net reproductive ability of mated pairs of the domestic fowl.

2. This index expresses the actual number of chicks produced by the mating and capable of living three weeks after hatching as a percentage of the maximum total number of chicks which it would be physiologically possible for the mating to produce during the time which it endures, each of these theoretical chicks being supposed to live to at least three weeks of age.

3. The values of this reproductive index are calculated for 1114 Barred Plymouth Rock matings, covering a period of nine years, and the data so obtained are treated biometrically.

4. For the strain of Barred Rocks used, and under the conditions of environment and management which obtained during the experiments, the reproductive index has a mean value of about 12 percent.

5. Net fertility, as measured by the reproductive index, is a rather highly variable character, agreeing in this respect with other purely physiological characters.

6. Reproductive ability, as measured by the index, diminishes with advancing age of the birds mated, having its maximum when each of the birds mated is from 10 to 14 months of age.

7. The decline in reproductive ability with advancing age is at a more rapid rate in the case of the males than in the case of the females.

8. The results above stated are to be understood as being limited, for the present, to the breed, strain, and circumstances, which furnished the data. How wide their generality may be is a matter yet to be investigated.

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THE RELATION OF YELLOW COAT COLOR AND BLACK-EYED WHITE SPOTTING OF MICE IN INHERITANCE

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CUÉNOT, STURTEVANT and MORGAN have all shown that a series of mutually allelomorphic forms of coat pattern exists in mice. There are four types of pattern in this series. These in order, from the most hypostatic to the most epistatic, are as follows: (1) *Non-agouti,* the ordinary "self" or "unticked" coat pattern. (2) *Agouti* or better *gray-bellied agouti,* the coat pattern seen in the ordinary wild house mouse. (3) *White-bellied agouti,* in which there is a decrease in brown and black pigmentation resulting in more yellow on the dorsal surface and white-tipped ventral hairs. (4) The final member of the series, the *yellow* coat pattern in which almost, if not all, the brown and black pigment of the coat has disappeared and is replaced by yellow.

It is easy to obtain mice homozygous for any of the three lower members of the series: non-agouti, gray-bellied agouti, and white-bellied agouti. No one, however, has yet obtained yellow mice which are homozygous. There is every reason to believe that the process of fertilization between two yellow-bearing gametes occurs (CASTLE and LITTLE 1910). The ratio of yellow to non-yellow young, however, makes it certain that the homozygous yellow zygotes do not reach a sufficiently advanced age to enable one to record them (CASTLE and LITTLE 1910, LITTLE 1911, DURHAM 1911, DUNN 1916). The size of litters when yellows are crossed *inter se* is also smaller than when yellows are crossed with any of the hypostatic types (CASTLE and LITTLE 1910, DURHAM 1911, DUNN 1916).

If we are to consider that allelomorphs occupy the same locus in the chromosome we are faced with a condition somewhat as follows. Four distinct stages occur in the restriction of brown and black pigment from the coat. The first three stages, though they cover a wide range of variation in extent of brown and black pigmentation, may all of them be obtained in a homozygous condition. The fourth step, however,

apparently so affects the individual that it can exist only when balanced in the zygote by one of the three lower steps. When both gametes contain the factor for this advanced restriction, the zygote perishes early in its existence.

In a most interesting paper given at the recent New York meeting of the AMERICAN SOCIETY OF ZOOLOGISTS, KIRKHAM has reported the discovery of disintegrating embryos in mice, where the "homozygous yellow" embryos should be found. These disintegrating embryos occur in number corresponding sufficiently well with the Mendelian expectation to make the evidence concerning the fate of the homozygous yellow zygote conclusive.

It is also of fundamental importance to note that in this case, a color factor has shown that it may be an active force in morphogenesis long before the embryo has formed any pigment whatever. This indicates clearly that *the function of a genetic factor may be entirely different at different stages in ontogeny.*

A similar condition exists in the case of "black-eyed white" spotting in mice. Independent in inheritance of either self coat or the ordinary piebald spotting, the black-eyed white factor produces, when acting on "self" mice, an individual with a small number of white spots, occasionally irregular in outline, and when acting on piebald mice, a white individual with pigmented eyes. Such black-eyed whites, however, do not breed true, but give a ratio of approximately one piebald to two black-eyed whites, when crossed *inter se* (LITTLE 1915).

Since these two peculiar results in mice stand as entirely distinct from any others obtained in the study of color factors in rodents it seemed worth while to investigate what relation, if any, they bear to one another.

For the purposes of explanation we may construct the following diagrams to show the separate behavior of the "yellow" and the "black-eyed white" factors in heredity.

Let Y equal the factor for "yellow" coat pattern, and y equal that for non-yellow (non-agouti, gray-bellied agouti, or white-bellied agouti, as the case may be). A heterozygous yellow may be represented by the factors Yy. Such an animal forms gametes Y and y. When two such yellows are crossed together, the following result is obtained:

Mating, $Yy \times Yy$
Gametes, Y and y Y and y
Zygotes, 1 YY, homozygous form which fails to continue development.
2 Yy, heterozygous yellow.
1 yy, non-yellow.

Similarly we may let *W* equal the factor for "black-eyed white" spotting, and *w* its absence. A heterozygous black-eyed white mouse would have the formula *Ww*. If two such animals were crossed *inter se* the following result would be obtained:

Mating, *Ww* × *Ww*
Gametes, *W* and *w* *W* and *w*
Zygotes, 1 *WW*, homozygous form which fails to develop.
2 *Ww*, heterozygous black-eyed whites.
1 *ww*, non-black-eyed white (ordinary piebald).

The interesting question to answer, if possible, is whether *Y* and *W* act in an identical manner and therefore are unable to exist in a single zygote. Further, if they are not identical, are they in any way related or are they entirely distinct physiologically and genetically.

All the "black-eyed white" mice used in the earlier experiments with this variety were non-yellow; i. e., gray-bellied agouti or non-agouti. This was shown by the fact that all of the piebald young produced by them were either gray-bellied agouti or non-agouti. We may then express the zygotic formula of the black-eyed whites as follows: *yyWw*, that is to say they were homozygous for "non-yellow" but heterozygous for the "black-eyed white" factor. The yellows used were entirely free from the "black-eyed white" factor and of course possessed the yellow factor in a heterozygous condition. Their formula would be *Yyww*. A cross between yellow and black-eyed white (non-yellow) mice would give the following result:

Yellow, *Yyww* × *yyWw*, black-eyed white (non-yellow)
Gametes, *Yw* and *yw* *yW* and *yw*
F_1 zygotes, (a) *YyWw*, yellow.
(b) *Yyww*, yellow.
(c) *yyWw*, non-yellow.
(d) *yyww*, non-yellow.

If the lethal action of *Y* and *W* is the same, the *YyWw* individuals comprising class (a) of the F_1 generation should be non-viable. The *Yw* and *yW* gametes might meet in fertilization, just as do two *Y* or two *W* gametes, and the resulting zygote might perish before attaining sexual maturity as do the *YY* and *WW* zygotes. If this happened, class (a) of the F_1 generation would form but fail to develop and the resulting ratio of yellow to non-yellow young in F_1 would be one to two, not one to one. The actual numbers of F_1 young observed were, yellows 76, non-yellows 81. On a 1 : 1 ratio the Mendelian expectation would be 78.5 : 78.5. If a 1 : 2 ratio was the correct explanation the numbers expected are

52 yellow, 105 non-yellow. There is no doubt that the equality ratio is the one approximated. This being the case, it is certain that the action of *y* and *W* is not identical.

The next matter of interest is to determine whether *any* relationship between *Y* and *W* exists, or whether they are entirely distinct from one another.

One possibility is that no fusion between a *Yw* and a *yW* gamete is possible. There might be a selective fertilization. If this is the case, the *Yw* gametes would always be met in fertilization by *yw* gametes producing *Yyww* zygotes. The *yW* gametes will always fertilize or be fertilized by *yw* gametes producing *yyWw* zygotes. *The result would be that F_1 yellow animals when crossed inter se or with piebald mice of any color should never give black-eyed white young.* The F_1 ratio would probably approximate one yellow to one non-yellow and we should have to resort to a breeding test and an F_2 generation to determine whether or not there was actual selective fertilization. As a possible modification of this idea of selective fertilization one might imagine that the combination *YyWw rarely* did form but that would mean that an occasional yellow F_1 animal would carry the *W* factor and give rise to black-eyed white young, when suitably mated. The exact ratio of such yellows to the more common *Yyww* type would be determined by the degree of antagonism between *Yw* and *yw* gametes which had to be overcome before their union was possible.

The remaining hypothesis is that *Y* and *W* although they act alike in their elimination of a zygote containing a double dose of either of them, are physiologically and genetically entirely distinct. If such were the case, the F_1 generation would have an equality ratio of yellows to non-yellows and approximately an equal number of *each* color would or would not carry the *W* factor.

Before turning to the experimental evidence on which the tests of these hypotheses are based, it may be profitable to review very briefly the main facts of the genetic behavior of black-eyed white mice. When black-eyed whites are crossed with self-colored, non-yellow mice, two sorts of F_1 animals are obtained. These I have described and figured in a previous paper (LITTLE 1915). I have called them type A and type B. Type A has a distinct spotted appearance. The head and hind quarters are commonly pigmented, but the trunk is usually unpigmented to a considerable degree. The spots of pigment in this position of the body are inclined to be small and irregular in outline in

contrast to the large and regular type of pigment patch seen in piebald mice and guinea-pigs. Type B is either entirely without white or else possesses a small amount of white on the ventral surface. Mice of this type are indistinguishable from the heterozygotes obtained when self-colored and piebald mice are crossed.

The type A animals all of them carry the *W* factor and produce a certain number of black-eyed white young when crossed *inter se* or with piebald animals. The type B animals never give black-eyed white young when crossed *inter se* or with piebalds. The actual numbers which I have obtained are as follows. These figures are a combination of those already reported (LITTLE 1915) with additional data from new crosses between self-colored dilute brown and black-eyed white (non-yellow) mice.

Black-eyed white × *self-colored:*
F_1 generation: Type A, 91, type B, 98.

Type A × *piebald:*

Observed:	Self-colored, 64;	type A, or spotted, 96;	black-eyed white, 42.
Expected:	*50.5*	*101.0*	*50.5*

Type B × *piebald:*

Observed:	Self-colored or type B, 94;	piebald, 101.
Expected:	*97.5*	*97.5*

If we represent the factor for black-eyed white spotting by *W* and its absence by *w*, and the factor for self coat by *S* and its allelomorph for piebald coat by s^p, we may represent the above-mentioned crosses as follows:

Mating: Black-eyed white $s^p s^p Ww$ × $SSww$ self-colored (non-black-eyed white)

Gametes: s^pW Sw
s^pw Sw

F_1 zygotes Ss^pWw, type A.
Ss^pww, type B.

Type A × piebald
$Ss^pWw \times s^p s^p ww$

SW, Sw, s^pW, s^pw } s^pw gametes

Zygotes
- Ss^pWw, type A
- Ss^pww, self or type B.
- $s^p s^pWw$, black-eyed white.
- $s^p s^pww$, piebald.

Type B × piebald
$Ss^pww \times s^p s^p ww$

Sw, s^pw } s^pw gametes

Zygotes Ss^pww, type B
$s^p s^pww$, piebald.

EXPERIMENTAL EVIDENCE FROM CROSSES BETWEEN YELLOWS AND BLACK-EYED WHITE MICE

In making these matings reciprocal crosses were made and gave identical results. Generally speaking it may be said at the outset that the F_1 generation animals possessed much less white than the F_1 animals in either of the former crosses between black-eyed white and self-colored mice. If the F_1 animals are classified into type A and type B mice according to previous methods the yellow $\times$ black-eyed white matings gave only 63 type A to 94 type B instead of the expected equality ratio of 78.5 to 78.5. If we analyze this generation more closely we find that among the non-yellow animals of the F_1 generation, there is an almost absolute equality of type A and type B animals. The exact figures are forty-one non-yellow type A and forty non-yellow type B. The yellow F_1 animals, however, give a decided preponderance of animals with no dorsal white, which are classed as type B. The actual figures in the yellows are twenty-two of type A and fifty-four of type B. Superficially the evidence appears to favor the idea of selective fertilization. A study of the F_2 generation, however, brings out certain extremely interesting facts which make any such assumption unnecessary.

If the type B animals obtained in the F_1 generation of the yellow—black-eyed white cross be tabulated according to the approximate percentage of the ventral surface which is unpigmented it will be noticed that many of the *yellow* type B animals have a distinctly greater amount of unpigmented ventral surface than do the type B non-yellows obtained in the same crosses.

Generation		Percentage of ventral surface white																	
		0	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	51-55	56-60	—	—	—	76-80	Total
F_1 yellow	Type B	29	8	8	2	3	1	0	0	1	0	1	0	0	0	0	0	1	54
F_1 non-yellow	Type B	31	8	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	40

Thus thirty-nine of the forty or 97.5 percent of the non-yellow type B animals have less than 6 percent of the ventral surface white while only one has just 6 percent. On the other hand among the yellow type B mice seventeen or 31.4 percent have 6 percent or more of the ventral surface white. Nine of these or 16.6 percent of the total yellows have a degree of whiteness not recorded in type B animals of any previous cross. Eleven of the seventeen yellow animals showing 6 percent or more ventral white have been tested by crossing *and have all shown that they are really type A individuals, not type B as they had been*

classified. If we remove these from the latter category and place them under type A where they really belong, we have the following result:

	Before correction	After correction	*Expected*
Yellow type A	22	33	*39*
Yellow type B	54	43	*39*
Non-yellow type A	41	41	*39*
Non-yellow type B	40	40	*39*

There is every reason to believe that if the other six yellows of a similar degree of whiteness to those tested had also been bred, a further addition to the yellow type A class and subtraction from the yellow type B class would be made, thus bringing the figures even closer to the expected results.

The corrected figures suggest that the proper hypothesis is one of complete independence between the factors for yellow and black-eyed white.

TEST MATIGS OF F_1 ANIMALS

Non-yellow type A animals of the F_1 generation were tested by crossing them with piebald mice. The details of such a cross have already been worked out above. The expectation is one piebald to one type A, to one self-colored or type B, to one black-eyed white. Classing the first two named types together, since at times no distinguishing breeding tests were made, we should expect two spotted, to one self-colored or type B, to one black-eyed white. The actual result was:

	Observed	*Expected*
Spotted	22	*22*
Self or type B	10	*11*
Black-eyed white	12	*11*
Total	44	*44*

The agreement between observation and expectation is striking. Yellow type A animals, among which the eleven tested mice first classed as type B are included, have been crossed with piebald non-yellows and have given a total of 165 young. The classes of young expected in this cross are exactly the same as in the previous cross with the exception that there is an equal chance of an animal being yellow or non-yellow in

color. The ratio expected then, is one self yellow or type B, to one black-eyed white (yellow), to two spotted yellows, to one non-yellow self or type B, to one black-eyed white (non-yellow) to two non-yellow spotted. The actual results follow:

	Observed	*Expected*
Yellow spotted	35	*42*
Non-yellow spotted	46	*42*
Yellow self or type B	31	*21*
Non-yellow self or type B	21	*21*
Yellow black-eyed white	4	*21*
Non-yellow black-eyed white	28	*21*
Total	165	*168*

If we tabulate this generation in a slightly different way we find that the expected figures are approximated more closely in the non-yellows than in the yellows.

	Yellow		Non-yellow	
	Observed	*Expected*	Observed	*Expected*
Spotted	31	*34*	46	*48*
Self or type B	35	*17*	21	*24*
Black-eyed white	4	*17*	28	*24*
Total	70	*68*	95	*96*

It is possible by a close examination of the yellow animals to reclassify certain animals on the basis of comparison with the F_1 generation and of direct breeding tests. Thus there are among the thirty-one yellows of the self or "type B" class, eleven which are identical in appearance with the F_1 "type B" yellows that upon breeding turned out to be type A. While all eleven have not been tested by breeding, several have, and seem to justify classifying these eleven animals under the spotted category as being type A in genetic constitution. After this change the figures read:

	Before change	After change	*Expected*
Yellow spotted	35	46	*34*
Yellow self or type B	31	20	*17*
Yellow, black-eyed white	4	4	*17*

The discrepancy between the observation and expectation in the yellow black-eyed white class is interesting but I believe only apparent. If the yellow and non-yellow spotted animals are tabulated according to the percentage of the dorsal surface which is pigmented it will be noticed that there are fourteen yellows that have less pigment than all but two of the non-yellow spotted mice. Ten of them have less than thirty per-

Generation	Percentage of dorsal surface pigmented									
	11-20	21-30	31-40	41-50	51-60	61-70	71-80	81-90	91-99	Total
Yellow spotted F_2	8	2	1	3	1	3	3	7	8	36
Non-yellow spotted F_2	1	0	1	0	4	10	6	11	9	42

cent of the dorsal surface pigmented and eight have twenty percent or less. Ordinarily non-yellow black-eyed whites have, as I have shown in my earlier paper, rarely more than 5 percent of the dorsal surface pigmented. Since, however, certain of the F_1 and F_2 type A yellows have distinctly more pigment than the corresponding variety of non-yellows, it seemed probable that the same might hold true in the case of yellow and non-yellow black-eyed whites. In order to test this question, several of the lightly pigmented spotted yellows were bred to non-yellow piebald mice. The uniform result when yellows with twenty percent or less of dorsal pigmentation are crossed with piebalds was the production of a nearly equal number of black-eyed white[1] and piebald young. This proves that they are genetically black-eyed white mice and are not, therefore, to be included in the "spotted" column. We may then properly remove the yellows with twenty percent or less dorsal pigmentation from the spotted class and place them in the class of yellow black-eyed whites as follows:

	Before change	After change	*Expected*
Yellow spotted	46	38	*34*
Yellow self or type B	20	20	*17*
Yellow black-eyed white	4	12	*17*
Total	70	70	*68*

Undoubtedly the separation of yellows at the twenty percent mark is arbitrary and it is certain from breeding tests of lightly spotted yellows of other more advanced generations that animals with as much

[1] Among the black-eyed whites are classed yellows resembling their parents, i. e., with 5-20 percent of the dorsal surface pigmented.

as forty percent of the dorsal surface yellow may breed like black-eyed whites. This means that if breeding tests had been made of the two yellows with between twenty and thirty percent dorsal pigmentation both of them would in all probability have joined the class of black-eyed whites and brought the figures even closer to the expectation.

We may now combine the yellows and non-yellows in one category, according to whether they are spotted, black-eyed white, or belong to the self type B class. When this is done the following figures are obtained:

	Observed	*Expected*
Total spotted	84	*82*
Total self type B	41	*41*
Total black-eyed white	40	*41*
Grand total	165	*164*

Breeding tests of yellow type B animals crossed with piebald non-yellows show that, as expected, they give four classes of young,—yellow and non-yellow self or type B and piebald yellows and non-yellows in approximately equal numbers.

	Observed	*Expected*
Yellow self type B	36	*35*
Non-yellow self type B	37	*35*
Yellow piebald	30	*35*
Non-yellow piebald	38	*35*
Total	141	*140*

Non-yellow type B animals crossed with piebald give essentially similar results, the yellow classes are, of course, lacking.

	Observed	*Expected*
Non-yellow self type B	43	*36*
Non-yellow piebald	29	*36*

Breeding tests of the back-cross animals produced in the above crosses have shown that all of the expected genetic classes of young occur. In view of this fact it appears fairly certain that the test of the relationship of the yellow and black-eyed white factors has been satisfactorily made.

SIZE OF LITTERS

CASTLE and LITTLE (1910) have called attention to the fact that litters from yellow × yellow matings are smaller on the average than litters from yellow × non-yellow matings. If the given explanation of the relationship of the yellow and black-eyed white factors is correct the litters produced by crossing together two yellow type A mice should be distinctly smaller than the litters produced when a piebald non-yellow. The reason for this will become apparent if we examine the types of zygotes formed when two yellow type A animals are intercrossed.

Mating: Yellow type A *YyWw* × *YyWw* yellow type A

Gametes: *YW* *YW*
Yw *Yw*
yW *yW*
yw *yw*

Zygotes as shown by the checker-board method.

1 *YW* *YW*	2 *YW* *Yw*	3 *YW* *yW*	4 *YW* *yw*
5 *Yw* *YW*	6 *Yw* *Yw*	7 *Yw* *yW*	8 *Yw* *yw*
9 *yW* *YW*	10 *yW* *Yw*	11 *yW* *yW*	12 *yW* *yw*
13 *yw* *YW*	14 *yw* *Yw*	15 *yw* *yW*	16 *yw* *yw*

Any zygote containing two "doses" of either the *Y* factor or the *W* factor is eliminated. This would remove zygotes Nos. 1, 2, 3, 5, 6, 9, and 11. Only nine of the sixteen original combinations can continue development and this would result in cutting the litters to nearly one-half their expected size. What actually happens may be seen from the following figures. Ten litters from yellow type A parents crossed *inter se* gave a total of thirty young or an average of three per litter. On the other hand nine litters of young produced by crossing yellow type A mice with non-yellow piebalds produced forty-five young or an average of five per litter. The figures though not extensive bear out the explanation of the relationship of the two factors as outlined above.

INCREASE OF PIGMENTATION IN YELLOW MICE

The fact that yellow type A and black-eyed whites potentially yellow

have considerably more pigment than the corresponding non-yellow varieties in the F_1 and F_2 generation suggests that the increase may be due to a darkening factor which coming in apparently with the *Y*-bearing gamete of the yellow race shows a marked tendency to remain coupled with yellow coat color in inheritance. Experiments have been started to determine whether the increased pigmentation of yellow animals is due to interaction of factors or to linkage of a darkening modifier as suggested above.

CONCLUSIONS

(1) The factors for yellow coat color and for black-eyed white spotting in mice are both physiologically and genetically distinct and independent from one another.

(2) Certain apparent indications of selective fertilization and abnormal Mendelian ratios are found not to hold after breeding tests of the animals in question have been made.

(3) Yellow F_1 animals of "type A" are distinctly more heavily pigmented than non-yellow animals of the same type and generation.

(4) "Black-eyed whites," potentially yellow, have from 6-20 percent more dorsal pigmentation than do the corresponding black-eyed whites potentially non-yellow.

(5) Until further evidence is collected, this increase in pigmentation may be considered as due either (a) to a modifying factor linked with yellow or (b) to an interaction between the factor *Y* and the factor *W* of a purely somatic nature.

(6) Litters produced from yellow "type A" parents crossed *inter se* are distinctly smaller than litters in which only one parent is of this variety. This reduction is probably due to the formation and subsequent death of zygotes having a double representation of either the *Y* or *W* factor or of both.

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DEFICIENCY[1]

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INTRODUCTORY SUMMARY

The general term "deficiency" is used to designate the loss or inactivation of an entire, definite, and measurable section of genes and framework of a chromosome. A case of deficiency in the X chromosome of *Drosophila ampelophila* occurred in September 1914, and has given rise to a whole series of correlated phenomena.[2] The first indication of this deficiency was the occurrence of a female which had failed to inherit from her father his sex-linked dominant mutant "bar",[3] though she inherited in a normal manner his sex-linked recessive mutant "white." This female, when bred, gave only about half as many sons as daughters, the missing sons, as shown by the linkage relations, being those which had received that X which was deficient for bar. This lethal action in-

[1] Contribution from the Zoölogical Laboratory of COLUMBIA UNIVERSITY, and the CARNEGIE INSTITUTION OF WASHINGTON.

[2] A brief account of deficiency was included in "Non-disjunction as proof of the chromosome theory of heredity." Genetics **1**: 1-52, 107-163, Jan.-Mar., 1916. A fuller account was read before the American Society of Naturalists at the meeting held at COLUMBIA UNIVERSITY on December 29, 1916.

[3] For information about the various mutants, and for an explanation of the terms and symbols used, see appendix, p. 456.

dicates that the deficiency mutation involved not simply the bar, but was also a deficiency for one or more genes necessary to the life of the fly. It was next found that the deficiency was extensive enough so that it included the locus for "forked," a recessive mutant whose gene lies in the X chromosome about half a unit from the locus of bar. A deficient-bearing female behaves as though haploid for forked, so that a $\frac{\quad}{f}$ female is forked although forked is a strict recessive. That the region between forked and bar was likewise affected, was demonstrated by the disappearance of crossing over between these loci in females having a deficient X. The deficiency mutation is thus proved not only to have affected a region of adjacent genes but also to have affected the framework of the chromosome on which crossing over must primarily depend. The maximum and the minimum lengths of the deficient region were measured by two genetic methods—by means of haploid tests and by means of linkage. The length of the chromosome as tested by linkage was found to be shorter than normal by an amount corresponding to the length of the deficient region. Unfortunately the stock of deficiency was lost before a cytological examination was made. The fact that the female with one X deficient for bar and the other X carrying bar $\left(\frac{\quad}{B'}\right)$ is an intermediate like the normal bar heterozygote, leads to the conclusion that the intermediate eye shape is due to the broadening action of genes outside the bar locus rather than to a broadening action of the normal allelomorph of bar (b'). Of the two alternative explanations of the nature of deficiency, viz., physical loss and complete inactivation, the loss view is perhaps slightly favored by the evidence. The origin of the dominant mutant bar can not be explained on the "presence and absence" hypothesis as due to the loss of an inhibitor, for the loss of the inhibitor region through deficiency does not give a result comparable to the bar mutation. Sex differentiation was not affected by the occurrence of deficiency; hence sex is determined by specific differentiators which are in some part of the X other than the region from forked to bar. This case of deficiency enables us to establish an identity between the actual localization of certain genes in the X chromosome and their positions as mapped by means of linkage.

THE OCCURRENCE OF THE DEFICIENCY FOR BAR

The exception which led to the discovery of the first case of deficiency occurred (September 25, 1914) among the offspring of an XXY wild-

type female heterozygous for eosin and for vermilion ($\frac{w^e}{v}$) which had been outcrossed to a white bar male ($\frac{w}{B'}$) of a pure stock (table 1). Bar is a dominant mutant, for which reason all

TABLE 1

The occurrence of the bar-deficient exception among the offspring of a $\frac{w^e}{v}$ ♀ *outcrossed to a white bar.* ♂.

	Regular ♀♀		Regular ♂♂				Exceptions by secondary non-disjunction		Exception by bar-deficiency
			$\frac{w^e}{v}$		$\frac{w^e}{+}$	$\frac{v}{+}$			
No.	w-w^eB'	B'	w^e	v	w^ev	+	+♀	wB'♂	w-w^e♀
546*	60	59	45	48	14	18	10	10	1

* This culture appeared in table 9, p. 23, of "Non-disjunction as proof of the chromosome theory of heredity," Genetics **1**: Culture 546 is not included in the summary of table 14, appendix.

the daughters were bar,—either white-eosin bar ($\frac{w^e}{w}$ $\frac{}{B'}$) or simply bar ($\frac{v}{w}$ $\frac{}{B'}$),—except one, which was white-eosin but *not bar* ($\frac{w^e}{w}$ $\frac{}{}$). That is, *she had inherited the white from her father but had not inherited his bar.* If the genes for all the sex-linked characters are transmitted to daughters by means of a common vehicle, viz., the single X chromosome, then so long as this X is intact and behaves as a unit there should be no chance for him to transmit some of his sex-linked characters without transmitting all of them, as occurred in this case.

The mutation responsible for the bar-deficiency occurred in the germ tract of the white bar male at or close to the maturation division; for he produced many regular daughters but only one with the bar gene deficient.

THE LETHAL ACTION OF DEFICIENCY

The white-eosin exceptional daughter, outcrossed to a wild male, gave (table 2) no bar offspring whatever,—a fresh proof of the deficiency of bar. The sons were of the two expected classes, eosin and white; white was thus again proved to have been transmitted. But the total of the

TABLE 2

The lethal result given by the white-eosin bar-deficient female of culture 546 when outcrossed to a wild male.

Culture	Daughters	Sons			
		$\frac{w}{w^e}$ —		$\frac{w}{w^e}$ | —	
No.		Dies	w^e	w	Dies
593	84	—	37	14	—

sons (51) was only about half the total of the daughters (84), and the white sons (14) were much fewer than the eosin (37). The culture which produced the bar-deficient exception (table 1) contained no such lethal, as is shown by the equality of the sexes (130♀ : 135♂) and by the equality of all contrary classes. The appearance of an inequality of sexes and of contrary classes indicates that whatever cause had removed or transformed the bar of the white bar chromosome was sufficiently damaging so that every male which received the deficient chromosome was unable to come to maturity. The deficiency was then not simply for bar but was also a deficiency for one or more genes whose normal action is essential to the life of the fly. The amount of linkage shown between the lethal effect and white is that expected on the view that the lethal change occurred at or near the bar locus. The white sons which did live were those which by crossing over in the mother ($\frac{w^e}{w}$ ——) had received from the eosin-bearing chromosome a normal piece to replace the damaged piece containing the region formerly occupied by bar and the vital allelomorphs.

STOCK OF DEFICIENCY

The possession of one deficient X (——) does not kill the female, which is saved by the action of the dominant vital allelomorphs carried by the other X. Half of the daughters of such a female receive this deficient X and repeat the genetic results of their mother. A stock of deficiency was kept by selecting in each generation the daughters containing the deficient X, and, as is the practice with all lethals, this selection was rendered certain by linkage.

THE INCLUSION OF FORKED IN THE DEFICIENT REGION

It now seemed probable that the X chromosome of that particular sperm which gave rise to the exceptional daughter must have lost[4] a fragment containing the bar gene and also one or more genes necessary to the life of the animal. If this were the true explanation the chromosome should be deficient for all the genes within a definite distance of bar. It was anticipated that the section might be long enough to include more known loci than bar and the vital allelomorphs, and accordingly systematic tests were carried out with the mutations whose genes lie in the neighborhood of bar. The male, having but one X chromosome, is normally haploid with respect to all sex-linked genes; those females which have one normal X and one X deficient in the locus for a particular gene should behave with respect to that gene as if haploid and not as if diploid. That is, any recessive character whose locus lies opposite the deficient region should show itself in such a heterozygous female in spite of the fact that the character is normally a strict recessive, for in the deficient region there should be no active allelomorph to dominate.

Accordingly the next step was to test females carrying the deficient X, by forked, by rudimentary, and by fused, these being the recessive mutants with loci closest to bar (see map p. 457). The expected result was obtained with forked. Females heterozygous for deficiency, when outcrossed to forked males, gave half of their daughters forked, though these daughters were only heterozygous for forked! These forked daughters were derived from that half of the eggs which retained the deficient X, as was proved by the lethal result, as well as by the linkage relations shown by these forked females when they were bred (tables 4-8). This evidence shows that the mutation which removed the gene for bar and the vital allelomorphs, involved not simply these genes but also the normal genes of a section of the chromosome extensive enough to include the locus for forked.

THE MAXIMUM AND THE MINIMUM LENGTHS OF THE DEFICIENT REGION AS MEASURED BY THE HAPLOID TESTS WITH THE RECESSIVES

In the case of the other recessives (rudimentary and fused) the result obtained with forked did not occur (tables 4 and 5, appendix), which shows that the maximum length of the deficient region is the interval be-

[4] Since the results expected from complete inactivation are practically indistinguishable from those due to physical loss, consideration of the inactivation alternative will be deferred to a special section.

TABLE 3

The inclusion of forked in the deficient region as shown by tests of deficient-bearing females ($\frac{w}{}$ $\frac{-}{}$) *by forked males.*

Culture	Daughters		Sons $\frac{w}{}$ $\frac{=}{}$			
			$\frac{w}{}$ $\frac{-}{}$		$\frac{w}{}$ $\frac{\|}{}$ $\frac{-}{=}$	
No.	*f!*	+	Dies	+	*w*	Dies
668	49	55	—	24	28	—
669	79	72	—	41	25	—
691	129	107	—	76	46	—
692	128	114	—	66	47	—
Total	385	348	—	207	146	—

tween and exclusive of these two recessives, which are the nearest unaffected characters on either side. This maximum length is about 4.4 units. These same tests together with the data from the origin of deficiency show that the minimum length is the interval between and including forked and bar (about half a unit). The test with fused, which lies nearer the end of the chromosome than forked and bar (see map p. 457), brought out another significant point, namely, that the deficient X had not lost the entire end of the chromosome, but rather was deficient for a section near the end, leaving the genetic materials unchanged beyond this region. By crossing over between the deficient region and fused, females have been obtained in which the deficient X carries fused in the normal piece beyond the deficient region (table 6, appendix).

THE ABSENCE OF CROSSING OVER WITHIN THE DEFICIENT REGION

It seemed probable that the lethal effect of deficiency was not simply due to the deficiency for forked and bar, but rather that the deficiency had affected all the loci between forked and bar, including one or more whose normal action was essential to the life of the fly. In general it is to be anticipated that cases of deficiency should act as lethals, since it seems probable that any very extensive piece includes a locus vital to the animal. Very small deficiencies might conceivably fail to include such a vital locus, and it was thought that such smaller sections of the forked-bar deficiency might be obtained by crossing over in a female carrying forked—bar deficiency in one X and forked and bar in the other X. ($\frac{\quad}{f \quad B'} \rightarrow \frac{\quad \mid B'}{f \mid -}$) In an effort to test this possibility 1716 males were raised, but these did not show any crossovers (table 14, ap-

pendix). Either all crossover males died because of having a lethal fragment of the deficient region, or there were no crossovers to live or die. In testing this question an experiment (table 9, appendix) was devised so that the crossovers among the females could be detected, there being no question that the crossover females would live. There were raised 3138 such females and not one of them was a crossover! In this number of females 16 were expected to be crossovers. It is evident that crossing over in the deficient region is abnormally low, if indeed there is any crossing over whatever in this region. This disappearance of crossing over from the deficient region is practically proof that the entire region was involved, a section between and including forked and bar and certain vital genes.

THE EXTENT OF THE DEFICIENT REGION MEASURED BY LINKAGE

It was found that crossing over in other parts of the deficient X was of approximately normal frequency (table 14, appendix), and this fact made it possible to find out more nearly the maximum length of the deficient region. The elimination of genes and of crossing over from the deficient region results in a shortening of the genetic chromosome by an amount equal to the length of the deficient region. How much closer together have rudimentary and fused been brought by the occurrence of deficiency? Normally there is 4.4 percent of crossing over between rudimentary and fused. When deficiency was present there was a drop of 0.7 unit in this value (table 10, ♀♀, and table 14). This drop of 0.7 unit is in agreement with the previous data which showed that the minimum length of the deficient region is 0.5 unit. Evidently, however, the deficient region does not extend much beyond the forked—bar section.

THE DOMINANCE RELATIONS OF BAR AND THE NATURE OF THE BAR-DEFICIENT MUTATION

The mutative process, of which the first detected effect was (1) the loss of the dominant bar gene, involved also (2) the loss of the normal allelomorph of the recessive mutation forked, whose locus is about half a unit distant from that of bar, and (3) the loss of certain vital allelomorphs, which, from the linkage relations probably occupied the section between forked and bar; and (4) the disappearance of crossing over from this same region, due presumably to the loss of the physical framework of the chromosome. All of these four distinct but correlated effects can therefore be met and explained by the single hypothesis of the physi-

cal loss of a definite section of chromosome which has been measured by two genetic methods. It seems unreasonable that one of these effects should be due to a cause different from that of the others but initiated at the same time, as, for example, that the apparent loss of the bar gene (B') should have been in reality due to a remutation to its original wild-type allelomorph (b'), a process which will in nowise account for the other observed changes which had their origin in the same region at the same time.

It will be recalled that the eye shape of the normal heterozygous bar female ($\frac{B'}{b'}$) is an intermediate between the narrow bar of the homozygote and the round eye of the wild female. It was found (table 7, appendix) that the female carrying bar in one X and bar-deficiency in the other ($\frac{\quad}{B'}$) was in somatic appearance like the normal bar heterozygote and not like the homozygous bar female. The fact that the bar male with its one X chromosome carrying a single bar gene has an eye practically as narrow as that of the homozygous bar female, shows that one bar gene is sufficient to produce a fully narrow eye. That the bar gene of the $\frac{\quad}{B'}$ female does not make her eye fully narrow must be due to some opposing action tending to broaden the eye. Since the other X is deficient for the bar locus this broadening action must be due to genes outside of the bar locus. In the broad-eyed $\frac{\quad}{b'}$ female there are two sets of such broadening genes but only one narrowing gene (B'), while in the narrow-eyed homozygous bar female there are two narrowing genes. The narrowness of the eye of the bar male can be ascribed to its having the same ratio of narrowing action (1 B' gene: 1 set of broadening genes) as has the narrow-eyed homozygous female (2 B' genes: 2 sets of broadening genes). Likewise the broadness of the normal heterozygous bar female is not due to the action of the b' gene, as formerly supposed, but is due to the half ratio of narrowing to broadening genes (1 B' gene: 2 sets of broadening genes).

The suggestion that the broad-eyed $\frac{\quad}{B'}$ females owed their broadness to being in reality $\frac{-b'}{B'}$ females, due to the occurrence of a crossover ($\frac{\quad}{b'} \rightarrow \frac{-\,|\,b'}{\cdot\,-}$) somewhere in the ancestry, is met by the evidence that in the ancestry opportunity to become such a crossover had been open to but a single female, and that she should be

such a crossover is incredible in view of the fact that in an experiment to test the amount of crossing over in the deficient region not one female in a total of over three thousand had proved to be a crossover.

The mutation responsible for deficiency may not have been, as so far assumed, the physical loss of a section of the chromosome; it may have been some kind of "inactivation," such as an internal rearrangement with change of properties, the loss of essential materials, or the addition of inhibiting agents. If inactivation is the explanation of deficiency then it must be complete inactivation; for in every case in which characteristics have disappeared, they have disappeared entirely. Thus, for example, the dominant bar gene retains no trace of its narrowing effect: the eye of a heterozygous female ($\frac{\quad}{b'}$) is wholly round, and the eye of the $\frac{\quad}{B'}$ female is no narrower than that of the regular heterozygote. In favor of the inactivation view may be cited the striking analogy with the Y chromosome: the Y, while cytologically of the same nature as other chromosomes, is genetically of little significance, as is proved by the fact that the effect of sex-linked genes in the male is in no case altered by anything in the Y, by the fact that the supernumerary Y's obtained through non-disjunction are without effect upon the visible characters, and by the lethal effect,—a fly having one or two Y's but no X being unable to live. Furthermore, there is no crossing over between Y and X even when the Y is in a female (XXY) in which there is certainly synapsis between Y and X and in which the occurrence of crossing over between the other chromosomes shows that the failure of crossing over in the case of the Y is due to a peculiarity of the Y itself. Since the Y offers a case of a chromosome inactive with respect to both the genetic materials and the framework, it becomes possible to suppose that the case of deficiency is an example of the same process that has produced the inactivation of the Y. That a piece of the Y chromosome has actually been substituted for the corresponding piece of the X seems impossible because such a substitution would involve three very improbable occurrences, viz., (1) crossing over in the male, (2) crossing over of the Y chromosome, and (3) a double crossover embracing a section only about a half unit long, while the shortest section in which a double crossover is known to have occurred is 13.5 units long.

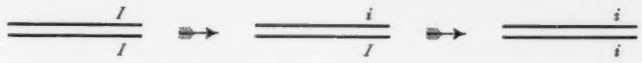
On the other hand, the "loss" view offers a more comprehensible solution for the fact that character genes different in nature but adjacent in position were affected by the one mutative change, and also for the fact that this one mutation affected, at the same time, the crossing over,

which seems to depend upon the framework of the chromosome rather than upon the character genes. As evidence that pieces may be lost bodily from chromosomes and that fragments may join together, there may be offered two distinct cases of "duplication" (unpublished), a phenomenon, the explanation of which seems to be that a section taken from the mid-region of one X has become attached to the end of the other X, its mate. The mid-region of the latter chromosome is represented twice, once in the normal location and again at the end. It seems probable that the first X, from which the duplicating fragment was taken bodily, would show the characteristics of deficiency, though in the two cases of duplication there was no evidence as to the fate of these terminal sections.

It seemed that a cytological examination might definitely settle this question, for on the loss view of deficiency the chromosome should be visibly shorter by an amount corresponding to the section lost. If the lost section were long enough, a difference in length between the two X's of a female having one deficient X should be observable. As stated in the beginning, the stock of deficiency was lost before the examination could be made. Several new cases which are possibly deficiencies in various parts of the X and even in other chromosomes have arisen, and it is hoped that combined cytological and genetic studies of these cases will make the subject clearer.

DEFICIENCY AND THE "PRESENCE AND ABSENCE" HYPOTHESIS

By following up the clue that a certain observed change might be due to the loss of a section of chromosome, we have been able to demonstrate a number of new and unusual facts which were predictable on that basis, and have thereby made it highly probable that the correct explanation has been found. This case of deficiency therefore constitutes the first valid evidence upon the question of "presence and absence." And it is significant to notice that the occurrence of the deficiency of a considerable section of genes has not brought to light any visible mutative changes in the way of dominants, contrary to what might well be expected on the presence and absence hypothesis. This is the more significant when it is recalled that the deficient region included the locus for bar,—a known dominant mutation. According to the presence and absence hypothesis the original appearance of the dominant bar character was due to the loss from the chromosome of an inhibitor, thereby allowing the normal narrowing effect of the remaining complex to assert itself.



Round Half narrow Narrow

Now, it should make no difference whether this inhibitor were lost by a special loss involving only the inhibitor or whether it were lost because of being situated in a particular section which itself became lost. In other words, the chromosome which is deficient for the region carrying the inhibitor should allow the occurrence of the same narrowing effect that is allowed by the simple loss of the inhibitor. In point of fact, the deficiency of the region in which the inhibitor must be hypothecated does not produce an effect like that of the mutation responsible for bar. For, the female carrying one deficient X and one normal X shows no narrowing of the eye shape, and likewise the female carrying one deficient X and one bar X is no narrower in eye shape than a normal heterozygous bar. Thus, in the only case which has a direct bearing on the presence and absence hypothesis, it is seen that the expedient of the loss of inhibitors to explain the origin of a dominant mutation is of no avail.

If, however, the appearance of the bar character were due to the creation of a new presence, then of course the loss of this presence by deficiency should restore the original condition; but that advocates of "presence and absence" have little liking for this type of explanation of the origin of a dominant is evident from the lengths they go in some recent expositions to avoid the vexed question of the origin of presences.

DEFICIENCY AND SEX DIFFERENTIATION

With non-disjunction the proof was complete that two X chromosomes determine a female and one a male. However, it has been suspected that the determiner of sex is not the "X-as-a-whole," but that in some definite part or parts of the X there are specific sex-differentiators. The case of deficiency favors this view; for, an XX individual having one deficient X is a *female,* normal in appearance and function. Two intact X's are not necessary for the production of a female; that is, sex-production is a function of some particular part of the X rather than of the X as a whole. Sex-differentiation was not affected by the occurrence of deficiency because the differentiators are in some region of the X other than the section from forked to bar.

THE REALITY OF THE CHROMOSOME MAPS

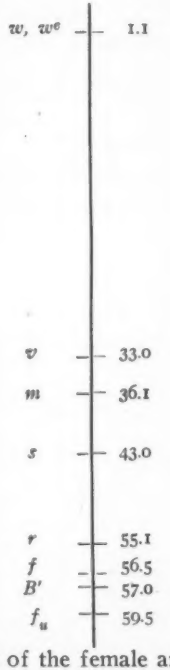
From the evidence of non-disjunction we know that the genes for the sex-linked characters are parts of the X chromosome. By means of

linkage studies we have been able to construct maps of the location of these sex-linked genes in a linear order which we believe to correspond to the linear structure of the chromosome. It has been objected that these maps may be only expressions of some "force" and that they do not correspond to an actual localization of genes along the chromosome. Deficiency furnishes the first direct evidence that the maps do correspond to a real localization of genes along the chromosome. That a single disturbing cause—the deficiency mutation—should exert a selective effect upon a certain few genes—bar, forked, vital allelomorphs—while leaving numerous other genes unaffected must be due to the possession by the few of a similarity either of properties or of location unshared by other genes. Nothing of the known properties of the dominant bar eye shape, the recessive forked bristle modification, and of the vital allelomorphs, are unique or suggest marked similarity to each other or dissimilarity between them and other sex-linked genes. *With regard to the location, however, there is independent and conclusive evidence from the linkage that these genes constitute a definitely localized and measurable section of the X chromosome.* The deficiency mutation was discovered because of its effect upon a single gene, viz., bar. It was then found to have affected at the same time one or more vital allelomorphs. When these vital allelomorphs are mapped according to the linkage shown, they are seen to occupy the region adjacent to bar. The argument from the location of forked within the deficient region is still stronger, for this location was detected and proved as the result of a deliberate search among those genes which had *previously been mapped closest to bar!* Nor does the evidence stop here, for not only did the deficiency mutation affect a section of adjacent genes but it also removed the crossing over from a definite section of chromosome. Now when the section from which the crossing over has been removed is compared upon the map with the section in which the genes are affected the two are seen to be identical. For a definite section of genes an identity has been established between the map and an actual distribution of genes.

APPENDIX

The sex-linked mutants referred to in this paper are: white eye color (w); eosin eye color (w^e), allelomorphic to white, and giving in females carrying white in one X and eosin in the other $\left(\frac{w}{w^e}\right)$, an intermediate eye color, "white-eosin compound"; vermilion eye color (v); miniature wings (m); sable body color (s); rudimentary wings (r);

forked bristles (f); bar eye shape (B'); fused venation (f_u); and deficiency for the region between and including forked and bar (—). Flies which show no mutant characters are said to be wild-type (+). The symbols included in parentheses do double duty, both to represent the genes for the mutants, and to tell the somatic appearance of flies (table headings, etc.); the small letters represent recessive mutants, and the primed capitals dominant mutants. The localization of these genes along the X chromosome as calculated from the linkage relations is given by the accompanying map[5] in which one unit of distance is one percent of total crossing over.



The two X chromosomes of the female are represented by two parallel lines with symbols showing the relative positions of the mutant genes involved ($\frac{w}{v}$), or more often by a single line, in which case the space above the line with its symbols represents one X and the space below, the other X ($\frac{w}{\quad v}$). The chromosome which has the mutant gene farthest to the left of those involved in the cross is arbitrarily

[5] From MORGAN and BRIDGES 1916, Carnegie Institution of Washington publication 237.

represented in the top space. The symbol $\frac{w^e}{\quad}\,|\,\frac{\quad}{v}$ is a contraction of , and denotes that, by crossing over in a female which carries eosin in one X and vermilion in the other, the two eggs, eosin vermilion and wild-type, are produced. The zygote arising from the egg represented by the upper space is always written in the column to the left in the double column beneath the crossover symbol; likewise the column to the right corresponds to the egg of the lower space. By following this convention, we may often omit from the tables the individual headings of the two included columns (e.g., see table 11). In place of the crossover symbol, it is often advantageous to use the "crossover formula" as in table 13. Thus, in the first case in table 13, $(\frac{w^e \quad\quad f}{vm \quad B^r})$, the "0" represents the sum of the flies that came from the two non-crossover gametes, eosin forked and vermilion miniature bar; the "1" represents the single crossovers between eosin and vermilion, that is, in the "first crossing over region"; the "1, 3 double crossovers" represent the flies resulting from the gametes $\frac{w^e}{\quad}\,|\,\frac{vm}{\quad}\,|\,\frac{f}{B^r}$; etc.

TABLE 4

The non-inclusion of rudimentary in the deficient region as shown by the tests of deficient-bearing females by rudimentary males.

Forked females $(\frac{w^e \quad\quad -}{\quad\quad f})$ *from culture 857, table 9A.*

No.	Daughters		Sons*			
			$\frac{w^e \quad -}{\quad f}$		$\frac{w^e}{\quad}\,|\,\frac{f}{-}$	
	+	r	Dies	f	$w^e f$	Dies
994	42	0	—	22	10	—
997	116	0	—	29	23	—
998	79	0	—	26	22	—
999	147	0	—	35	32	—
Total	384	0	—	112	87	—

* When females are heterozygous for deficiency and for forked, crossing over in the deficient region (if it occurs) should give the two contrary classes, non-forked sons and forked sons $(\frac{\quad\quad -}{\quad f}\,|\,\frac{\quad}{-})$. These sons should live only if they escaped retaining a lethal fragment of the deficient origin. The observed total lack

of any non-forked sons among the offspring is then additional evidence that no crossovers occur, or that these crossovers die. However, the forked crossovers would not be distinguished from the forked non-crossovers, and therefore this sort of evidence is equivalent only to a half amount as compared with the data of tables 6 and 8. Accordingly, in table 14, the data from males on crossing over in the deficient region includes all the males of tables 6 and 8, but only half the males of tables 4, 5, 7, 9, and 9 A.

TABLE 5

The non-inclusion of fused in the deficient region as shown by the tests of deficient-bearing females by fused males.

Forked females ($\frac{w^e \quad -}{\quad f}$) *from culture 857, table 9A.*

No.	Daughters		Sons			
			$\frac{w^e \quad -}{\quad f}$		$\frac{w^e \quad \mid f}{\quad -}$	
	+	f_u	Dies	f	$w^e f$	Dies
988	118	0	—	37	13	—
989	71	0	—	27	16	—
991	206	0	—	50	44	—
993	175	0	—	40	27	—
Total	570	0	—	154	100	—

TABLE 6

The insertion of fused into the intact region beyond the deficient region.

No.	♀♀	♂♂ *			
		$\frac{\quad f_u}{f \quad B'}$		$\frac{\quad \mid f_u}{f \quad B'}$	
		Dies	fB'	Dies	$fB'f_u$
1755	134	—	58	—	1
2176	30	—	11	—	1
Total	164	—	69	—	2

* These males are not included in the summary of table 14 as showing the amount of crossing over between deficiency and fused, for there might be other like cultures (table 8) which failed of detection because no crossover occurred. A crossover value calculated from such incomplete data could only be regarded as a maximum value.

TABLE 7

Tests of the relation between deficiency and bar by outcrossing deficient-bearing females (from culture 668, table 3) to bar males.

No.	♀♀	♂♂			
		$\frac{w^e \quad -}{\quad f}$		$\frac{w^e \mid f}{- \mid -}$	
	B'	Dies	f	$w^e f$	Dies
749	60	—	17	11	—
751	24	—	8	3	—
754	52	—	19	15	—
Total	136	—	44	29	—

TABLE 8

The non-occurrence of crossover sons of deficient-bearing mothers heterozygous for forked and bar ($\frac{\quad}{f \quad B'}$).

No.	Daughters	Sons			
		$\frac{\quad \quad}{f \quad B'}$		$\frac{- \quad B'}{f \mid -}$	
		Dies	fB'	B'	f
1200	51	—	33	—	—
1313	81	—	35	—	—
1752	97	—	25	—	—
1753	79	—	43	—	—
1754	114	—	64	—	—
1756	54	—	38	—	—
1757	41	—	26	—	—
1981	130	—	57	—	—
1981r	39	—	21	—	—
2177	41	—	31	—	—
Total	727	—	373	—	—

TABLE 8A

Mothers heterozygous for sable also ($\frac{s \quad f \quad B'}{\quad\quad}$).

		$\frac{s \quad f \quad B'}{\quad\quad}$		$\frac{s \quad \quad}{f \quad B'}$		$\frac{s \quad f}{\quad B'}$	
		sfB'	Dies	Dies	*fB'*	*sf*	*B'*
1145	124	50	—	—	12	—	—
1758	78	17	—	—	3	—	—
1759	38	17	—	—	2	—	—
1764	107	35	—	—	10	—	—
1765	97	31	—	—	4	—	—
1766	36	12	—	—	4	—	—
1767	113	49	—	—	11	—	—
Total	593	211	—	—	46	—	—

TABLE 9

The non-occurrence of crossover daughters when deficient-bearing mothers heterozygous for bar ($\frac{\quad\quad}{B'}$) *were backcrossed to forked males.*

No.	Daughters				Sons			
	$\frac{\quad\quad}{B'}$		$\frac{\quad B'}{\quad\quad}$		$\frac{\quad\quad}{B'}$		$\frac{\quad B'}{\quad\quad}$	
	f	*B'*	*fB'*	+	Dies	*B'*	*B'*	+
2222	77	90	—	—	—	76	—	—
2223	69	66	—	—	—	49	—	—
2224	60	64	—	—	—	69	—	—
2234*	75	64	—	—	—	69	—	—
2235	77	81	—	—	—	61	—	—
2236	51	50	—	—	—	34	—	—
2238	51	71	—	—	—	71	—	—
2239	68	53	—	—	—	65	—	—
2240	82	61	—	—	—	49	—	—
2241	54	59	—	—	—	46	—	—
2246	77	70	—	—	—	58	—	—
2260	85	82	—	—	—	113	—	—
2261	42	32	—	—	—	11	—	—
2264	86	73	—	—	—	81	—	—
2265	62	90	—	—	—	76	—	—
2286	48	49	—	—	—	56	—	—
2290	93	127	—	—	—	111	—	—
2316	79	106	—	—	—	86	—	—
2320	37	70	—	—	—	77	—	—
Total	1273	1358	—	—	—	1258	—	—

TABLE 9 A

Mothers heterozygous for eosin also ($\frac{w^e \quad ---}{B'}$).

					$\frac{w^e \quad ---}{B'}$		$\frac{w^e \quad B'}{---}$		$\frac{w^e \quad ---}{---\quad B'}$	
					Dies	B'	w^e	Dies	w^eB'	+
857	60	83	—	—	—	48	24	—	—	—
858	108	121	—	—	—	48	47	—	—	—
859*	60	75	—	—	—	40	29	—	—	—
Total	228	279	—	—	—	136	100	—	—	—

* In each of the cultures 2234 and 859 a sterile forked male (XO) appeared, due to primary non-disjunction.

TABLE 10

The crossing over in deficient-bearing females heterozygous for rudimentary and fused ($\frac{r \quad f_u}{---}$).

No.	Daughters				Sons*					
	$\frac{r \quad f_u}{---}$		$\frac{r \quad ---}{---\quad f_u}$		$\frac{r \quad f_u}{---}$		$\frac{r \quad ---}{---\quad f_u}$		$\frac{r \quad ---}{---\quad f_u}$	
	rf_u		r	f_u	rf_u	Dies	Dies	f_u	r	Dies
1437	17	80	0	2	20	—	—	0	1	—
1438	43	89	1	1	41	—	—	1	2	—
1599	27	51	3	1	40	—	—	2	0	—
1600	45	66	2	3	56	—	—	2	0	—
1601	38	59	1	1	37	—	—	1	0	—
1619	21	37	1	3	12	—	—	0	0	—
1696	18	37	0	2	16	—	—	0	0	—
1697	15	21	2	0	8	—	—	1	0	—
1700	21	26	1	3	10	—	—	0	2	—
Total	245	466	11	16						
1775			80		16	—	—	1	1	—
1778			31		10	—	—	0	0	—
1779			67		16	—	—	0	1	—
1905			31		15	—	—	0	0	—
1906			31		19	—	—	0	1	—
2106			100		32	—	—	4	1	—
2107			67		14	—	—	0	1	—
2175			56		31	—	—	1	1	—
Total			463		393	—	—	13	11	—

* The males of table 10 are not included in the summary of table 14, because of the difficulty of calculating the true amount of crossing over when a lethal (deficiency) and a poorly viable mutant (rudimentary) are both present. Ordinarily the poor viability of a mutant has little effect upon the apparent amount of crossing over, because, in each pair of contrary classes, the relative smallness of the class in which the non-viable mutant occurs is counterbalanced by the relative largeness of the contrary class in which its viable normal allelomorph occurs. This is the case with the females

of table 10. But when a lethal also is present (as in the case of the males of table 10), the lethal kills one of each pair of contrary classes and hence certain classes are relatively too low (as, in table 10, the non-crossover class rudimentary fused and the "2" crossover class rudimentary), while certain other classes are relatively too high (as the "1" crossover class fused). That the males of table 10 are in agreement with the females is evident after a correction has been made for the disturbance due to the unbalanced non-viability. The females of table 10 gave the interval between rudimentary and fused as 3.7 units, instead of the normal 4.4, a decrease of .7 unit due to the deficient region. The percentage expectation for the males on this basis (the distance from rudimentary to the deficient region = 1.4, the length of the deficient region = .7 unit, and deficiency to fused = 2.3) is "0" = 96.3, "1" = 1.4, and "2" = 2.3. These percentages become "0" = 94.24, "1" = 3.11, and "2" = 2.65 if 44 percent of the rudimentary and 88 percent of the fused zygotes hatch. The observed percentages are "0" = 94.24, "1" = 3.12, and "2" = 2.64. The assumed percentages of viability of rudimentary and of fused are in agreement with the results of other experiments in which these mutants are involved.

TABLE 11

The linkage of white and deficiency.

No.	Daughters	$\frac{w}{-}$		$\frac{w}{-\ \vert\ -}$	
632	166	—	34	14	—
642	123	—	40	35	—
645	264	—	82	53	—
647	216	—	59	35	—
731	58	—	32	12	—
Total	827	—	247	149	—

TABLE 12

The linkage of sable and deficiency.

$\frac{s}{-}$ *females backcrossed to sable forked males.*

No.	Daughters				Sons			
	$\frac{s}{-}$		$\frac{s}{-\ \vert\ -}$		$\frac{s}{-}$		$\frac{s}{-\ \vert\ -}$	
	s	*f*	*sf*	+	*s*	Dies	Dies	+
1024	34	39	5	8	31	—	—	5
1025	27	26	5	7	21	—	—	6
1026	34	39	6	9	33	—	—	6
1201*	41	52	8	11	49	—	—	3
1242	73	73	8	14	72	—	—	7
1244	29	20	5	3	27	—	—	6
Total	238	249	37	52				
867	111				58	—	—	11
900	81				35	—	—	5
975	88				44	—	—	10
1120	83				41	—	—	11
Total	363				411	—	—	70

* In culture 1201 a sterile sable forked male (XO) appeared, due to primary non-disjunction.

TABLE 13

Incidental linkage data not involving deficiency.

Genes	No.	Classes with respect to crossing over						
$\frac{w^e \quad f}{vm \quad B'}$	862	0	1	2	3	1, 3	2, 3	1, 2, 3
		45	30	2	23	8	1	1
$\frac{w^e \quad f}{B'}$	861	0				1		
		42				22		
$\frac{v \; m \quad B'}{f}$	863	0			1		2	
		68			2		31	
$\frac{s}{f}$	864	0				1		
		102				27		
	865	123				22		
	866	92				18		
	868	80				9		
	869	137				15		
	870	139				25		
	871	75				15		
	872	131				19		
	873	77				9		
	885	44				7		
	1235	152				27		
	1243	267				39		
	Total	1419				232		
$\frac{r}{B'}$	2263	0				1		
		153				3		
$\frac{r \quad f_u}{B'}$	2242	0			1		2	
		111			3		8	
	2243	66			1		2	
	2245	24			1		2	
	2262	15			1		0	
	2267	192			0		7	
	2287	114			3		5	
	2289	131			2		5	
	2291	132			1		3	
	2317	112			3		1	
	2318	157			4		5	
	2319	85			2		1	
	2352	94			1		3	
	Total	1233			22		42	
$\frac{r}{f_u}$	1174	0				1		
		49				0		
	1175	20				0		
	1207	110				3		
	1208	91				5		
	1209	101				3		
	1281	106				3		
	1282	47				1		
	1308	113				4		
	Total	637				19		

TABLE 13 (Continued)

Genes	No.	Classes with respect to crossing over	
		0	1
	860	379	1
$\frac{f}{B'}$	1021	163	3
	1022	163	1
	Total	705	5
$\frac{B'}{f_u}$		0	1
	2244	100	4

TABLE 14

A summary of the linkage data of this paper.

Genes	Total	Crossovers	Percent
w v	110	39	35.4
w m	110	41	37.3
w —	1562	625	40.0
w f	174	78	44.8
w B'	174	78	44.8
v m	211	6	2.8
v f	211	66	31.3
v B'	211	66	31.3
m f	211	64	30.3
m B'	211	64	30.3
s —	1314	205	15.6
s f	1651	232	14.0
r B'	1453	25	1.7
r f_u	1953	83	4.3
r f_u	738	27	3.7
f B'	980	5	0.5
f B'	♂♂ 1716	0	0.0
	♀♀ 3138	0	0.0
B' f_u	1401	46	3.3

DOMINANCE OF LINKED FACTORS AS A MEANS OF ACCOUNTING FOR HETEROSIS[1]

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A stimulation resulting from hybridization in both plants and animals has long been recognized. The increased growth as the result of crossing is so common an occurrence that it is probably familiar to everyone who has made any hybridization experiments.

This stimulation, variously spoken of as "hybrid vigor," stimulus due to heterozygosis, heterosis, etc., was clearly established as an organic phenomenon by the abundant cases cited by early investigators such as KÖLREUTER (1766), GÄRTNER (1849), DARWIN (1877) and FOCKE (1881), as well as a large number of other investigators at that time and an increasingly large number since then. The important investigations in recent times (EAST 1908, 1909; SHULL 1908, 1909, 1910, 1911; EAST and HAYES 1912) are so familiar that it is not necessary to do more than mention them.

Concrete explanations as to the cause of these results have not accompanied the accumulation of facts. Various hypotheses have attempted to account for the results, but they have been little more than outlines of the problem.

The valuable contributions of EAST (1908, 1909) and of SHULL (1908, 1909, 1910, 1911) established the fact that continued inbreeding is not a process of continuous degeneration but that the reduction in the amount of growth is due to the isolation of unlike biotypes differing in the amount of growth attained at normal maturity. Together with this isolation of biotypes there was a loss of a stimulation which was assumed to be derived in some way from crossing. This decrease of vigor becomes less after continued inbreeding and to all appearances ceases as complete homozygosis is approached. This stimulation has been shown to be correlated more or less closely with the degree of heterozygosity. The whole subject has been ably presented and discussed by EAST and

[1] Contribution from the CONNECTICUT AGRICULTURAL EXPERIMENT STATION and from the Bussey Institution of HARVARD UNIVERSITY.

HAYES (1912). A quotation from this paper (pp. 36 and 37) presents the matter as it stands at present:

"The hypotheses in regard to the way by which the act of fertilization initiates development are numerous, but since they are entirely speculative it is not necessary to discuss them here. The only conclusion that seems justified is that they are not immediately psychological or vitalistic in nature. LOEB's remarkable researches prove this. But whatever may be the explanation of the means by which the process is carried out, the statement can be made unreservedly that the heterozygous condition carries with it the function of increasing this stimulus to development. It may be mechanical, chemical, or electrical. One can say that greater developmental energy is evolved when the mate to an allelomorphic pair is lacking than when both are present in the zygote. In other words, developmental stimulus is less when like genes are received from both parents. But it is clearly recognized that this is a statement and not an explanation. The explanation is awaited."

KEEBLE and PELLEW (1910) first suggested a concrete explanation to account for the results of this nature which they obtained with peas. Two varieties of garden peas, as grown by them, each averaged from 5 to 6 feet in height. The F_1 grown from this cross averaged from 7 to 8 feet in height, 2 feet taller than either parent. A result of this kind is comparable to heterosis. The F_2 was put into four classes: one class containing plants as tall as the F_1, two classes of semi-tall plants similar in height to the two parents, and one class of dwarfs shorter than either parent. The two classes of semi-tall plants, similar in height, were differentiated in the same manner as the two parents; one had thick stems and short internodes, the other had thin stems and long internodes. Other differences helped to distinguish the two classes of equal height. The number of plants falling into these four classes agreed closely with the expectation from a di-hybrid ratio where two factors showing dominance were concerned, giving a 9:3:3:1 ratio.

The writers assumed two factors to be concerned: one producing thick stems, the other long internodes. These factors they designated *T* and *L*. One of the parental varieties was medium in height because it possessed one of these factors, e.g., that for thick stems, but lacked the other. Such a plant had the formula *TTll*. The other variety was of medium height because it lacked this *T* factor but possessed the factor for long internodes, and was given the formula *ttLL*. Both of these factors showed dominance over the allelomorphic condition; hence the F_1 was taller than either parent because both factors were present together. Whether or not later investigations have justified the interpretation that KEEBLE and PELLEW have placed on the data as explaining height of

their peas makes no material difference to the discussion here. Taken as it stands, it is a beautiful illustration of the way in which dominance may increase a character in F_1 over the condition of either parent.

Curiously enough, this explanation has never been considered an adequate one or in any way essentially related to the universal phenomenon of heterosis. This hypothesis of dominance accounting for heterosis, as outlined by KEEBLE and PELLEW, has two objections which have up to the present been considered insurmountable.

The chief objection has been that, if heterosis were due to the dominance of a greater or less number of factors governing the amount of development, it would be possible in generations subsequent to the F_2 to recombine in one homozygous race all of the factors resulting in large growth and, conversely, the negative condition in another homozygous race. In other words, it would be possible to obtain one strain having all of the dominant factors, and another with all of these dominant factors lacking. Both of these races should be homozygous, hence self-fertilization should not result in less vigorous progeny. The completely recessive race should be below the parents in its power for development, as the F_1 and the complete dominant were above the parents. That all of these supposedly necessary corollaries are not supported by the facts is well known.

Both SHULL (1911) and EAST and HAYES (1912) have considered this objection to be valid. A quotation (p. 39) from the latter makes their position on this point clear.

> "KEEBLE and PELLEW (1910) have recently suggested that 'the greater height and vigor which the F_1 generation of hybrids commonly exhibit may be due to the meeting in the zygote of dominant growth factors of more than one allelomorphic pair, one (or more) provided by the gametes of one parent, the other (or others) by the gametes of the other parent.' We do not believe this theory is correct. The 'tallness' and 'dwarfness' in peas which KEEBLE was investigating is a phenomenon apparently quite different from the ordinary transmissible size differences among plant varieties. Dwarf varieties exist among many cultivated plants, and in many known cases dwarfness is recessive to tallness. It acts as a monohybrid or possibly a dihybrid in inheritance, and tallness is fully dominant. Varietal size differences generally show no dominance, however, and are caused by several factors. Transmissible size differences are undoubtedly caused by certain genetic combinations (EAST 1911), but this has nothing to do with the increase of vigor which we are discussing. The latter is too universal a phenomenon among crosses to have any such explanation. Furthermore, such interpretation would not fitly explain the fact that all maize varieties lose vigor when inbred."

Another objection to the hypothesis of dominance has been raised by

EMERSON and EAST (1913). In this publication it is said that, if the effect of heterosis were due to dominance, the distribution of the F_2 individuals would be unsymmetrical in respect to characters in which heterosis was shown in F_1. This follows from the familiar Mendelian expectations where there is dominance and any number of factors is concerned. For the purpose of illustrating this point let us take the case of height of peas already cited. In the F_2 population a distribution of the individuals in respect to height is, theoretically, 9 tall plants (with both factors present), 6 medium-tall plants (3 with one factor + 3 with the other), and one short plant (with both factors lacking).

Similar asymmetrical distributions in F_2 would occur with any number of factors (if there were no other facts to be taken into consideration), as seen from the figures given in table 1 modified somewhat from those given by BAUR (1911, p. 63).

In any case of a size character similar to height of peas with any number of factors, the plotting of the number of individuals in F_2 occurring in the classes given in row B in table 1 would give an asymmetrical distribution. This is on the assumption that the individual having the greatest number of dominant factors present (whether in the simplex or duplex state) would attain the greatest development of the size character.

In the vast amount of data accumulated upon the inheritance of quantitative characters no such tendencies toward an asymmetrical distribution is evident in the majority of cases recorded. In EMERSON and EAST's paper, referred to, dealing with quantitative characters in maize, and in HAYES's publication (1912) dealing with the same type of characters in tobacco, the distributions in F_2, where heterosis is shown in F_1, are all considered to be of the type of normal frequency distributions. If any skewness is shown by any of these it is too slight to suggest the types of curves obtained by plotting the figures in table 1, B.

It is perfectly evident that the two objections raised against the hypothesis of dominance as a means of accounting for heterosis, as outlined by KEEBLE and PELLEW, and as it has been considered up to the present, are valid. But both these objections to dominance as an interpretation of heterosis have failed to take into consideration the fact of linkage.

Abundant evidence is fast being accumulated[2] to show that characters are inherited in groups. The different theories accounting for this link-

[2] It is unnecessary to give references to the convincing results obtained by MORGAN, BATESON, and their collaborators, as well as to those obtained by many others whose work is of great importance if not so extensive.

TABLE 1

Distribution of F_2 individuals when each character shows complete dominance and each has a visible effect.

Number of factors in which the F_1 is heterozygous		Distribution of the individuals	Total number in the population
1	A	3 : 1	4
	B	3 : 1	
	C	1 : 0	
	D	1 : 1	
2	A	9 : 3 : 3 : 1	16
	B	9 : 6 : 1	
	C	2 : 1 : 0	
	D	1 : 2 : 1	
3	A	27 : 9 : 9 : 9 : 3 : 3 : 3 : 1	64
	B	27 : 27 : 9 : 1	
	C	3 : 2 : 1 : 0	
	D	1 : 3 : 3 : 1	
4	A	81 : 27 : 27 : 27 : 27 : 9 : 9 : 9 : 9 : 9 : 3 : 3 : 3 : 3 : 1	256
	B	81 : 108 : 54 : 12 : 1	
	C	4 : 3 : 2 : 1 : 0	
	D	1 : 4 : 6 : 4 : 1	
n	A	$3^n : 3^{n-1} : 3^{n-1} : 3^{n-1} : 3^{n-1} : 3^{n-2} : 3^{n-2}$: etc. .... : 1	$(2^n)^2$
	B	$1(3^n) : D(3^{n-1}) :$ $D(3^{n-2})$: ..... etc. .... : 1	
	C	n : $n-1$: $n-2$: ..... etc. ... $n-n$	
	D	1 : ... etc. = coefficients of the expanded binomial $(a+a)^n$ ..... : 1	

A, The distribution into the visibly different categories. B, The distribution into categories with different numbers of dominant factors present (either in a homozygous or heterozygous condition). C, The number of dominant factors in which the categories differ. D, The number of visibly different categories with the same number of dominant factors present.

age of characters make no essential difference in the use to which these facts will be put here. It is only necessary to accept as an established fact that characters are inherited in groups and that it is these groups of factors which Mendelize. The chromosome view of heredity, as developed by MORGAN and others (1915), will be used because it gives a means of representation in a simple, graphical manner.

The increasing complexity of Mendelism points very strongly to the probability that the important characters of an organism are determined by factors represented in all or most of the chromosomes or linkage groups. This idea has been proposed by EAST (1915) and seems to be in accord with the facts. If this view is approximately correct, and if it

may also be assumed that, in addition to the factors which differentiate varieties, many different factors may bring about the same visible effect, then it is possible to meet the two objections raised against dominance as a means of accounting for heterosis.

As an illustration of what is meant by different factors bringing about the same visible effect, an example may be taken in which one variety of plants grows to an average height of six feet because of one set of factors, and another variety grows to approximately the same average height but attains this height through the operation of a different set of factors. This is comprehensible when it is remembered that height is only an expression of a plant's power to develop. Hereditary factors which affect any part of the plant may indirectly determine height. Direct proof as to the essential correctness of this assumption, i.e., of different factors producing the same somatic effect, is at hand in the cases of duplicate genes producing the same morphological result in *Avena sativa* (NILSSON-EHLE 1909) and *Bursa bursa-pastoris* (SHULL 1914), as well as the other cases of duplicate genes reported by NILSSON-EHLE (1908) and EAST (1910).

The widespread occurrence of abnormalities and other characters detrimental to the organism's best development is well known in both the plant and animal kingdoms. This is especially true in naturally cross-pollinated species of plants. It may be taken for granted that no one variety has all of these unfavorable characters nor, on the other hand, has it all the favorable characters. For the most part each variety possesses a random sample of the favorable and unfavorable characters. There are differences between varieties in their power for development, however, just as there are differences in superficial characters. Some varieties of plants grow taller than others; some grow faster; some produce more seed. But, on the average, most of the varieties of a species tend to grow to about the same extent, however much they may differ in superficial characters.

If, for the most part, these favorable characters are dominant over the unfavorable (if normalities are dominant over abnormalities) it is not necessary to assume complete dominance in order to have a reasonable explanation of the increased development in F_1 over the average of the parents or any subsequent generation. It is in F_1, and in F_1 only, that the maximum number of different factors can be accumulated in any one individual.

Because of linkage it is impossible to recombine in any one individual in later generations any greater number of characters in the homozygous

condition than were present in the parents if the factors were distributed uniformly in all of the chromosome pairs. Possible exceptions to this statement will be discussed later. This view of the situation explains why the effects of heterozygosis result in a greater development in F_1 than in the parents, and not less. Why should crossing not have resulted in a depressing or indifferent effect instead of a stimulating one, according to previous views?[3] It also makes it seem probable that the effects of heterozygosis remain throughout the life of the sporophyte, even through innumerable asexual generations. Furthermore, it will be shown that no skewness in the distribution of F_2 is expected.

Let me submit in the form of a concrete illustration the abstract view that I have tried to present in the preceding paragraphs. A purely hypothetical case will be assumed, in which two homozygous varieties of plants, having three pairs of chromosomes, both attain approximately the same development as represented by any measurable character. This development will be considered to amount to 6 units, 2 of which are contributed by each chromosome pair. One of these varieties, which will be called "X," attains this development because of factors distributed in the three pairs of chromosomes. Any number of factors may be chosen, but, for the sake of simplicity, only three in each chromosome will be employed. These are numbered 1, 3, 5; 7, 9, 11; and 13, 15, 17; in the following diagram, each different in its contribution to the plant's development. The other variety, "Y", develops to an equal extent in the character measured, and this development will also be considered to amount to 6 units. It attains this same development, however, by a different set of factors distributed in the three chromosomes, numbered 2, 4, 6; 8, 10, 12; and 14, 16, 18. It is also assumed that these 6 factors are fully as effective in the $1n$ condition as in the $2n$ condition, i.e., show perfect dominance. It will be seen from the diagram that the F_1 develops to twice the extent of either parent, because there are present here 18 different factors (in the $1n$ condition), whereas the parents have only 9 (in the $2n$ condition). In the diagram, any other factorial complex common to both varieties is ignored. The development of the parents of 6 units and of the F_1 of 12 units is additional to that afforded by this common factorial complex.

Following this hypothetical case into the F_2 generation by selfing or

[3] Crosses between plants not closely related do result in no greater development than the parents and in many cases much less than the parents. This is because characters which are widely dissimilar are unfavorable to the organism's best development when acting together.

breeding together these F_1 plants, the theoretical results given in table 2 are obtained.

Summing up the results of this tabulation, it will be found that eight plants are homozygous and have the same development as either parent, i.e., of six units. Eight plants are heterozygous in all three chromosome

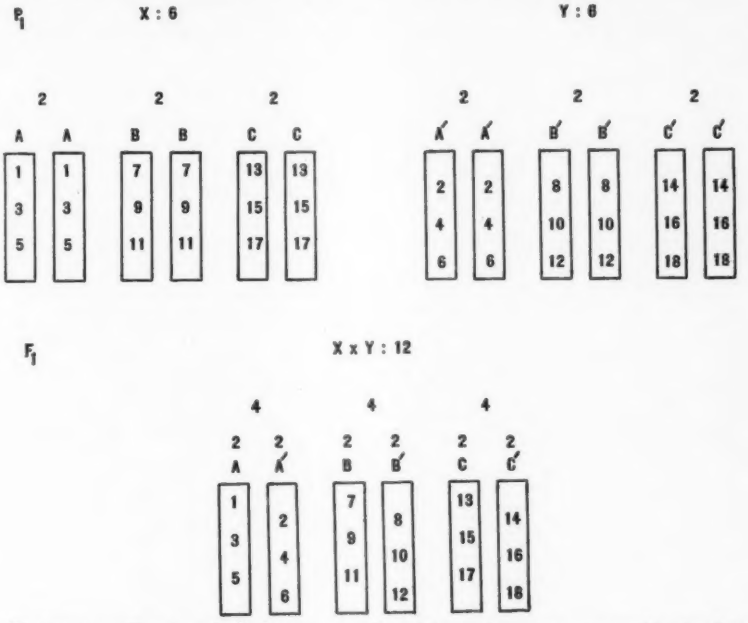

DIAGRAM 1.—To show how factors contributed by each parent may enable the first generation of a cross to obtain a greater development than either parent.

pairs and have the same amount of growth as F_1, i.e., of twelve units. The remaining 48 plants fall into two equal-sized groups developing to eight and ten units respectively. In other words, the distribution is symmetrical, and this symmetry remains, however many chromosomes are concerned.

Furthermore, it should be noted that the mean development of F_2 is nine units, which is an excess above the parents of just half of the excess of the F_1 over the parents. In other words, the extra growth derived by crossing the two varieties has diminished 50 percent. In F_3 from a random sample of F_2, it can be shown that this excess again diminishes 50 percent, so that the effect is only 25 percent as great in F_3 as in F_1,

TABLE 2

Composition of a tri-hybrid in F_2 according to Mendelism, and the development which each individual attains depending upon the number of heterozygous chromosomes contained and thereby the total number of different factors present.

Number of individuals in each category	Categories	Contribution of each chromosome paif	Total development
1	A A B B C C	2 + 2 + 2	6
2	A A′ B B C C	4 + 2 + 2	8
2	A A B B′ C C	2 + 4 + 2	8
2	A A B B C C′	2 + 2 + 4	8
4	A A′ B B′ C C	4 + 4 + 2	10
4	A A B B′ C C′	2 + 4 + 4	10
4	A A′ B B C C′	4 + 2 + 4	10
8	A A′ B B′ C C′	4 + 4 + 4	12
1	A A B B C′C′	2 + 2 + 2	6
2	A A B B′ C′C′	2 + 4 + 2	8
2	A A′ B B C′C′	4 + 2 + 2	8
4	A A′ B B′ C′C′	4 + 4 + 2	10
1	A A B′B′ C C	2 + 2 + 2	6
2	A A B′B′ C C′	2 + 2 + 4	8
2	A A′ B′B′ C C	4 + 2 + 2	8
4	A A′ B′B′ C C′	4 + 2 + 4	10
1	A′A′ B B C C	2 + 2 + 2	6
2	A′A′ B B′ C C	2 + 4 + 2	8
2	A′A′ B B C C′	2 + 2 + 4	8
4	A′A′ B B′ C C′	2 + 4 + 4	10
1	A′A′ B′B′ C C	2 + 2 + 2	6
2	A′A′ B′B′ C C′	2 + 2 + 4	8
1	A′A′ B B C′C′	2 + 2 + 2	6
2	A′A′ B B′ C′C′	2 + 4 + 2	8
1	A A B′B′ C′C′	2 + 2 + 2	6
2	A A′ B′B′ C′C′	4 + 2 + 2	8
1	A′A′ B′B′ C′C′	2 + 2 + 2	6
64 Total			

Distribution of the F_2 individuals according to the development attained.

Classes	6	8	10	12	= 4	Number of classes
Frequency	8	24	24	8	=64	Total population

and so on in subsequent generations. This is in accord with the mathematical prediction made by EAST and HAYES (1912), to which actual data obtained from maize roughly approximate, as shown by JONES (1916).

The development attained by any individual in table 2 is correlated with the number of heterozygous factors present. This has been main-

tained by all recent writers on the subject as a rough description of the facts as obtained in actual experiments.

When different numbers of chromosomes are concerned, according to this scheme, the number of individuals in the different classes making up the whole F_2 population is given in table 3.

In any F_2 distribution there are as many individuals heterozygous for all factors (duplicating F_1 individuals) as there are individuals homozygous for all factors concerned in the original cross (two duplicating the parents; the remaining forming new homozygous combinations). The remaining individuals fall into a symmetrical distribution between these two end classes. The theoretical figures for any F_2 distribution in which n Mendelizing units are concerned can be obtained by taking the coeffi-

TABLE 3

Distribution of the individuals in F_2 according to the number of heterozygous chromsomes pairs they contain.

Number of chromosome pairs in which the F_1 is heterozygous	Classes with different number of heterozygous chromosome pairs and the number and ratio of individuals in these classes						Total number of individuals in the population
	0	1	2	3	4	5	
1	2 1	2 1					4
2	4 1	8 2	4 1				16
3	8 1	24 3	24 3	8 1			64
4	16 1	64 4	96 6	64 4	16 1		256
5	32 1	160 5	320 10	320 10	160 5	32 1	1024
n	2^n 1	..... etc. .....$2^n \times$ (coefficients) .....2^n ..... etc. ..... coefficients of the expanded binomial $(a+a)^n$.....1					$(2^n)^2$

cients of the expanded binomial $(a + a)^n$ and multiplying these by 2^n, as shown in table 3. Since the expanded binomial is used to illustrate a normal frequency distribution, there can be no question as to the symmetry of the F_2 distributions if the diagrammatic scheme outlined is, in this respect, a description of the actual facts.

In the preceding purely diagrammatic representation of the way in

which dominance may account for the effects of heterozygosis, perfect dominance was assumed. Such an assumption is neither justified nor desirable. Many theoretical explanations of the inheritance of quantitative characters are based on exactly the converse assumption, i.e., that factors in the *1n* condition have just half the effect that they have in the *2n* condition.

In the development of an organism, however, all types of factors are concerned, both qualitative and quantitative. Partial dominance in qualitative characters is a normal occurrence. The concensus of opinion at the present time is that there may be, in reality, no cases of perfect dominance. In those cases in which the heterozygote cannot be distinguished from the pure dominant, it is assumed that the similarity is only apparent and not real. The heterozygote merely approaches the condition of the dominant type more or less closely. However much it may be true that perfect dominance rarely or never occurs, the fact and universality of partial dominance can hardly be denied.

In this connection it should be realized that the difference between the heterozygote and the recessive type in many cases is one of *kind,* while the difference between the heterozygote and the dominant type is one of *degree.* A good illustration of this point is found in the case of albinism in maize. Plants heterozygous for the factor (or factors) determining the production of chlorophyll cannot be distinguished from normal green plants—a case of apparently complete dominance. If there is in reality a difference between these heterozygous and homozygous normal green plants, although not apparent, that difference is very slight as compared with the difference between the heterozygote and the abnormal recessive. In the former case the difference, if there is any, is quantitative. The heterozygote may not have as much chlorophyll as the normal homozygote. In the second case the difference is qualitative. The heterozygote has chlorophyll; the recessive has none. This is a difference which determines the life or death of the organism.

All the evidence at hand leads to a seemingly logical conclusion, one necessary to the conception of dominance as an explanation of heterosis, which is, that *many factors in the 1n condition have more than one-half the effect that they have in the 2n condition.* Whether or not this is a logical conclusion and one that is justified by the facts remains to be seen. It certainly has the advantage of being more definite and comprehensible than the assumptions previously made (SHULL 1911; EAST and HAYES 1912), that factors in the heterozygous condition stimulate development by virtue of their being in that condition, without showing in any way why this should be so.

There is abundant evidence to show that many abnormal characters exist in a naturally cross-pollinated species and that they are recessive to the normal condition. In maize innumerable examples can be cited. In addition to the complete lack of chlorophyll already mentioned, there are also other chlorophyll factors which distinguish yellowish-green plants from normal green plants, just as there are cases of both conditions in other plants, e.g., Pelargonium (BAUR 1911). By inbreeding, strains of maize are isolated which are dwarf; some are sterile; some have contorted stems; some fasciated ears. Some are more susceptible to the bacterial wilt disease, and still others have brace roots so poorly developed that they cannot stand upright when the plants become heavy. It is unnecessary to mention more examples, because their occurrence in many kinds of material is familiar to everyone. All the characters cited are recessive, either completely or to a large degree, to the normal condition. More than one of these unfavorable characters may be present together in one inbred strain. No one strain so far known has them all.

Crossing many of these strains of maize together produces perfectly normal F_1 plants. They are normal because the factors which one strain lacks are supplied by the other, and conversely. Because more of the favorable characters are present when the strains are united in F_1 than in either parent, the F_1 is naturally able to attain a greater development. This effect is heterosis.

In the preceding diagrammatic illustration of the way in which heterosis may be brought about it was assumed that all factors had equal effects, that they were evenly distributed in the chromosomes, and that there were no crossovers. This is probably far from describing all the actual conditions. All deviations from this uniformity add to the complexity of the problem. It remains to be seen whether or not the assumption of dominance as an explanation of heterosis will not meet all or most of the requirements raised by all these complicating factors. It is only necessary to consider that a large number of factors is concerned, and that those factors are in most cases fairly evenly distributed among all the chromosomes, and that, in the main, crossovers in some places are balanced by crossovers in others.

Crossing over also provides a means of understanding why certain homozygous individuals (and varieties) may possess a greater number of desirable characters than others. Exceptionally good individuals might be formed by crossing over in heterozygotes occurring in such a manner that all, or a large number of, desirable characters would be combined together eventually in one individual. Such a condition, ac-

cording to the laws of chance, would be exceedingly rare, which is well in accord with the facts.

Without going into all the possibilities which this viewpoint opens up, it is only necessary to say that a way is offered to meet the objections which have been raised against the conception of dominance as a means of accounting for the facts of heterosis as so far known.

There is still the possibility that there may be a stimulus derived from crossing quite apart from hereditary factors. The view presented here simply coördinates the existing knowledge of heredity so as to give a comprehensible view of the way in which heterosis may be brought about.

SUMMARY

1. The phenomenon of increased growth derived from crossing both plants and animals has long been known but never accounted for in a comprehensible manner by any hypothesis free from serious objections.

2. The conception of dominance, as outlined by KEEBLE and PELLEW in 1910 and illustrated by them in height of peas, has had two objections which were: *a*. If heterosis were due to dominance of factors it was thought possible to recombine in generations subsequent to the F_2 all of the dominant characters in some individuals and all of the recessive characters in others in a homozygous condition. These individuals could not be changed by inbreeding. *b*. If dominance were concerned it was considered that the F_2 population would show an asymmetrical distribution.

3. All hypotheses attempting to account for heterosis have failed to take into consideration the fact of linkage.

4. It is shown that, on account of linked factors, the complete dominant or complete recessive can never or rarely be obtained, and why the distributions in F_2 are symmetrical.

5. From the fact that partial dominance of qualitative characters is a universal phenomenon and that abnormalities are nearly always recessive to the normal conditions, it is possible to account for the increased growth in F_1 because the greatest number of different factors are combined at that time.

6. It is not necessary to assume perfect dominance. It is only necessary to accept the conclusion that many factors in the $1n$ condition have more than one-half the effect that they have in the $2n$ condition.

7. This view of dominance of linked factors as a means of accounting for heterosis makes it easier to understand: *a*, why heterozygosis should have a stimulating rather than a depressing or neutral effect; and *b*, why

the effects of heterozygosis should operate throughout the lifetime of the individual, even through many generations of asexual propagation.

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SEX DETERMINATION IN *ANTHOTHRIPS VERBASCI*

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The mullein thrips is a species that was formerly supposed to reproduce only sexually. Both sexes are abundant; fully formed spermatozoa are produced by the male; and copulation may frequently be observed in nature. Two years ago, however, it was discovered (SHULL 1915) that virgin females also produced offspring. At the time of publication of the paper cited the sex of such parthenogenetically produced offspring was unknown. It could not be stated, therefore, whether the parthenogenesis of Anthothrips was of the aphid type, in which both sexes develop from parthenogenetic eggs; or of the honey-bee type, in which fertilized eggs produce females, unfertilized eggs males; or of a third type unlike any species now known.

The experiments here described were designed to solve the problem of the relation of parthenogenesis to sex in this species.

VIRGIN FEMALES PRODUCE ONLY MALES

The experiments that showed that virgin females produce only sons were of two kinds. These two kinds differed only in the time elapsing between the beginning of the experiment and the removal of the parents from the host plant. In some experiments the parents were removed before the first offspring became adult, in others not until adult offspring were present.

Experiments in which the parents were removed while the oldest offspring were still immature

Virgin females were procured by rearing pupae in isolation until they emerged as adults. The sex was ascertained by placing the live insect in a drop of water under a supported cover glass, and examining with a microscope. Only the females were retained. These were placed on young mullein plants that had been reared from seed under cover, and

were protected by lantern globes closed at the top with closely woven cloth. In some experiments only one female was placed on a single plant, in others a number of females were put together on the same plant. All these females were removed before the first offspring became adult. The offspring subsequently removed are recorded in table 1.

TABLE 1

A record of the sex of the offspring of virgin females of Anthothrips verbasci, in experiments in which the parents were removed before any of the offspring became adult.

Number of experiment	Number of females used as parents	Date of beginning of experiment	Date of removal of parents	Offspring	
				Males	Females
93	1	Aug. 28, 1914	Apr. 12	1	0
95	2	Aug. 30, 1914	Apr. 12	18	0
99	5	Sept. 11, 1914	Dec. 5	63	0
100	7	Sept. 14, 1914	Dec. 5	9	0
101	7	Sept. 16, 1914	Apr. 12	8	0
102	9	Sept. 16, 1914	Dec. 5	22	0
129	1	July 5, 1915	July 29	6	0
131	3	July 9, 1915	July 29	68	0
132	3	July 9, 1915	July 29	34	0
133	3	July 12, 1915	July 29	48	0
134	5	July 12, 1915	July 29	60	0
135	5	July 12, 1915	July 29	57	0
145	4	July 14, 1915	July 30	39	0
146	2	July 17, 1915	July 30	18	0
147	5	July 19, 1915	July 30	79	0
149	7	July 19, 1915	July 30	32	0
150	7	July 19, 1915	July 30	53	0
151	8	July 22, 1915	July 30	32	0
165	3	July 29, 1915	Sept. 21	38	0
170	5	July 29, 1915	Sept. 22	3	0
Totals	92			688	0

There is abundant proof in these experiments that unfertilized eggs produce males.

Experiments in which the parents were not removed until the first offspring were adult

These experiments were conducted in every way like those recorded in table 1, except that the parents remained on the host plant until some of the offspring were adult. When adults were subsequently removed from the plant, they may or may not have included the parents, depending on

whether the latter survived. The sex of the thrips removed from such plants is stated in table 2.

TABLE 2

A record of the sex of individuals of Anthothrips verbasci taken from plants on which virgin females had previously been placed. The virgin females were left on the plant until some of the offspring were adult, and may be included in this list with the offspring.

Number of experiment	Number of females used as parents	Date of beginning of experiment	Date of removal of parents	Offspring	
				Males	Females
102a	9	Dec. 5, 1915	June 9	15	3
148	5	July 19, 1915	Sept. 20	61	1
152	5	July 24, 1915	Sept. 20	19	3
153	5	July 24, 1915	Sept. 20	45	3
154	5	July 28, 1915	Sept. 20	47	5
155	6	July 28, 1915	Sept. 20	19	5
167	3	July 29, 1915	Sept. 21	28	2
168	5	July 29, 1915	Sept. 21	85	1
169	5	July 29, 1915	Sept. 22	61	2
177	4	July 30, 1915	Sept. 22	63	1
179	7	July 30, 1915	Sept. 23	12	2
Totals	59			455	28

While some females appear among the adults obtained from these plants, the number of females is in no case greater than the number of female parents with which the experiment was started. It is to be assumed, then, in harmony with the experiments of table 1, that the males in table 2 are the offspring, the females are the mothers.

From the above experiments, in both tables, it is sufficiently well established that unfertilized eggs produce only males.

FEMALES THAT MATE MAY PRODUCE BOTH SEXES

In a series of experiments females were either induced to mate in captivity, or were observed to mate in nature, and were then transferred to mulleins in the greenhouse and kept under cover. In each experiment the female alone was placed on the host plant, and she was removed before any of her offspring became adult. These offspring, on becoming adult, were removed and their sex ascertained. In some experiments they were collected from the host plant only once, in others several times. But in every experiment examination of the offspring ceased before any of their offspring (grandchildren of the original female) could have become adult. The records are certainly, therefore, those of the imme-

diate children only. In some experiments both daughters and sons were produced, in others only sons. Tables 3 and 4 show the sex of the offspring in these experiments, grouped for convenience according to the results.

TABLE 3

A record of the sex of the offspring of females of Anthothrips verbasci which were known to have mated, and subsequently produced both daughters and sons.

Number of experiment	Date of beginning experiment	Offspring	
		Males	Females
97	Sept. 3, 1914	18	2
117	July 5, 1915	52	4
119	July 5, 1915	6	2
120	July 5, 1915	33	3
125	July 5, 1915	5	3
138	July 12, 1915	5	2
139	July 12, 1915	51	7
140	July 12, 1915	2	1
143	July 12, 1915	23	6
144	July 12, 1915	32	7
159	July 28, 1915	31	8
Totals		258	45

TABLE 4

A record of the sex of the offspring of females of Anthothrips verbasci which were known to have mated, and subsequently produced only sons.

Number of experiment	Date of beginning experiment	Offspring	
		Males	Females
118	July 5, 1915	2	0
126	July 5, 1915	3	0
137	July 12, 1915	39	0
141	July 12, 1915	16	0
142	July 12, 1915	43	0
Totals		103	0

In harmony with the results shown in table 1, it is to be assumed that the males in table 4 were produced from unfertilized eggs, notwithstanding their mothers had mated. The occurrence of females among the offspring in table 3 and the production of nothing but males in table 1 indicate that fertilized eggs yield females, at least in part. The paucity of females may be partly accounted for by the fact that the females

mated only once, whereas in nature they may be observed to mate repeatedly.

Support for the conclusion that fertilized eggs yield females, at least in part, and unfertilized eggs always males, is found in the offspring of mixed lots of males and females. Two such lots were maintained for a time to provide a stock of thrips for use in experiments, and to ascertain what would be the sex ratio in the greenhouse under conditions as favorable as possible to repeated mating. Table 5 gives the sex of the adults collected from these two plants at intervals, each collection including all the adults present on the date named.

TABLE 5

Showing the sex ratio of the offspring produced by a mixed lot of males and females of Anthothrips verbasci under conditions favorable to repeated mating.

Number of experiment	Date of beginning experiment	Dates of collecting adult offspring	Offspring	
			Males	Females
203	June 28, 1916	Sept. 9, 1916	6	0
		Oct. 4, 1916	18	8
		Oct. 23, 1916	16	0
204	June 28, 1916	Sept. 9, 1916	1	2
		Sept. 30, 1916	0	3
		Oct. 15, 1916	0	2
		Oct. 24, 1916	25	43
		Nov. 23, 1916	15	7

These experiments are cited here as showing that when males are present, females appear among the offspring. The sex ratio and its significance is referred to elsewhere.

ALTERATION OF THE SEX RATIO IN FAMILIES OF FEMALES THAT MATE ONCE

From certain theoretical considerations discussed below, it was important to ascertain the sex ratio of the offspring of a female that had mated, and whether that sex ratio is altered if subsequent matings are prevented. Females that were known to have mated were placed alone upon mulleins. Before any of their offspring became adult, they were transferred to new plants. From this second plant the offspring were removed when they became adult, care being taken not to collect so late that any of the grandchildren might be included. In table 6 the sex ratio of the collection from each plant in these experiments is given.

The occurrence of females on the first plant of each experiment, and their absence from the second plant in most cases, is explained on the

TABLE 6

A record of the sex ratio of the offspring of single females of Anthothrips verbasci, known to have mated, the offspring being collected at intervals as they became adult.

Number of experiment	Date of beginning experiment	Dates of collecting adult offspring	Offspring	
			Males	Females
117	July 5, 1915	Sept. 19,* 1915	37	4
		Sept. 21, 1915	15	0
139	July 12, 1915	Sept. 20,* 1915	26	7
		Sept. 22, 1915	25	0
143	July 12, 1915	Sept. 20,* 1915	7	6
		Sept. 22, 1915	15	0
		Oct. 14, 1915	1	0
144	July 12, 1915	Sept. 20,* 1915	21	6
		Sept. 22, 1915	11	1
159	July 28, 1915	Sept. 21,* 1915	22	8
		Oct. 14, 1915	9	0

* Must have been mature much earlier. Were on the first plant to which the female was transferred.

assumption that the spermatozoa were all allowed to fertilize eggs at first, and that subsequent eggs were unfertilized.

Another experiment not included in table 6 because performed in a slightly different way, bears upon the same theoretical point, and is here recorded separately. In experiment 94, a virgin female and a male were placed together on a plant December 16, 1914. Copulation was not witnessed, but the results indicate that mating occurred. The first offspring became adult shortly before June 18, 1915, on which date all adults were removed. These proved to be 9 males and 27 females, and they may or may not have included the original parents. On June 29 another collection of adult offspring was made, comprising 53 males and 60 females. Further collections were made, but since the grandchildren may have had time to mature before these later lots were collected, the sex ratio of the later collections is of no value in this connection. In the figures given it is worthy of note that the females are in the majority, distinctly so in the first collection. The importance of this fact is indicated elsewhere.

DISCUSSION

From the experiments described it was early established that unferti-

lized eggs of *Anthothrips verbasci* produce only males. I also conclude that fertilized eggs produce only females, but was not forced to this conclusion until consideration of the above experiments left no other assumption probable. By analogy with the honey-bee, it was to be expected that, if parthenogenetic eggs yielded males, fertilized eggs would yield only females. But another analogy with the honey-bee, the degeneration of the male-producing spermatozoa, could not be established. The close relationship of the thrips to the Hemiptera on the one hand and the Orthoptera on the other, lead one to expect two types of spermatozoa, respectively male-producing and female-producing, in the thrips. Moreover, casual observations made upon poorly fixed material showed, in some cell divisions, what appeared to be a lagging chromosome, though I have not verified this observation on better material. However, if two types of spermatozoa are produced, analogy with the honey-bee requires the male-producing type to degenerate. This degeneration I have been unable to see in sections, though the minuteness of the object may account for my failure.

If sex in the thrips is not determined in the same way as in the honey-bee, there seemed but one probable alternative. It appeared possible that unfertilized eggs gave rise to males, and fertilized eggs to both males and females, depending on which type of spermatozoön fertilized the egg. The fact that no case of this kind is yet on record did not warrant the rejection of the hypothesis without consideration.

In the absence of any cytological evidence, the major part of the experiments described above were performed to test the correctness of the two hypotheses just outlined.

If the sex of thrips is determined in the same way as that of the honey-bee, the sex ratio may be anything. If, on the other hand, fertilized eggs yield males as well as females, in equal numbers, because there are two types of spermatozoa, the males must always predominate; for, to the fifty percent of males hatching from fertilized eggs must be added those developing from unfertilized eggs. The latter view also requires that there be two kinds of male, one from parthenogenetic and one from fertilized eggs.

The sex ratio among the offspring of impregnated females, as found in the greenhouse, usually showed a preponderance of males. Tables 3, 5, and 6 afford abundant illustrations. Certain exceptions, however, are crucial. Experiment 94 showed that a female which had every opportunity to mate produced, among her first offspring, 27 females and 9 males. A later collection comprised 60 females and 53 males. Like-

wise, in experiment 204 (table 5), which was started with a mixed lot of males and females, the first four collections of offspring showed a preponderance of females. The marked excess of females in these two cases seems to exclude the hypothesis that males arise from all parthenogenetic and half the fertilized eggs, because by that hypothesis there could never be, except by rare chance, a large majority of females. Furthermore, no evidence has been obtained that there are males of two kinds.

It seems necessary to conclude, therefore, that sex in this species of thrips is determined as in the honey-bee. Unfertilized eggs always produce males, fertilized eggs always females. Moreover, there is, as in the honey-bee, some means of withholding the spermatozoa, so that unfertilized eggs may be laid even while spermatozoa are present. Evidence that this is true is found in experiment 144 (table 6). The offspring produced on the first plant, which were collected September 20, included a large proportion of males. Yet, from the second plant, to which the mother was transferred July 30, one female and eleven males were taken September 22. The one female was taken as a pupa on that date and was allowed to emerge as an adult in the laboratory. The spermatozoön which fertilized the egg from which she developed must have been withheld while at least 21 earlier eggs were laid without fertilization.

This statement of the method of sex determination in thrips is made for only one species, *Anthothrips verbasci.* It is not improbable that it is true for some other species; but it can hardly be applied to species in which there are very few males, and certainly not to those in which there are no males. In *Thrips tabaci,* for example, males are rare (SHULL 1914); likewise in *Anaphothrips striatus* in certain regions (HINDS 1902). In these species, and others like them in this respect, rarity of males at any given time should, if sex were determined as in the mullein thrips, result in the parthenogenetic development of most of the eggs laid; and hence in an abundance of males in the next generation. The long periods of time over which the sex ratio of such species has been observed, preclude the possibility of a fluctuation as extreme as that mentioned. In *Thrips tabaci,* therefore, and in *Anaphothrips striatus* in regions where males are rare, the occasional males must arise through some change in the eggs (perhaps in their maturation), as in the aphids and phylloxerans.

That *Anthothrips verbasci* is not, however, the only thrips in which sex is dependent upon fertilization, is indicated by peculiarities of the sex ratio which were formerly very puzzling. In *Chirothrips manicatus,*

a species living on timothy, bluegrass and other grasses, the sex ratio has been observed (SHULL 1914) to fluctuate enormously within short periods of time. At Douglas Lake, Michigan, this species was observed at intervals in a small island of timothy in a thicket of bushes and small trees. As Chirothrips is not a ready flier, and shows little inclination even to crawl, the phenomenon about to be described can hardly have been due to migration. In this island of timothy the adult specimens collected were at first mostly females. Then adults practically disappeared for a time. A week or two later adults were again fairly abundant, but nearly all were males. A similar change in the sex ratio has since been observed in the outskirts of Ann Arbor, and I am told by Mr. C. B. WILLIAMS that he has seen the same occurrence in England. In the light of the experiments on *Anthothrips verbasci,* I think we may interpret the peculiar changes in the sex ratio of *Chirothrips manicatus.* The mothers of the adults collected were probably impregnated, and the first offspring were mostly females. For some reason mating did not occur again, and subsequent eggs developed parthenogenetically, producing males. Chirothrips is not as easy to rear under guarded conditions as Anthothrips, and experiments intended to verify or refute the above hypothesis failed. The only other explanation of the peculiarities of the sex ratio in Chirothrips that seems plausible is that perhaps the males require longer to develop than the females. From my experience with other insects I should expect just the reverse. I assume, therefore, that sex in *Chirothrips manicatus* is probably dependent on fertilization, just as in *Anthothrips verbasci.*

One more question is raised, but not answered, by the results of the experiments with Anthothrips. Why are the females more abundant than males in nature, whereas in the insectary the males have been found to be in the majority? It may be that the conditions of the greenhouse are not favorable to repeated copulation. Or the males may be short-lived, a fact which would not be detected in the experiments, since adults were usually still young when collected. Or the males may migrate more readily than the females. These hypotheses could readily be tested by experiment.

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SOME APPLICATIONS OF MATHEMATICS TO BREEDING PROBLEMS

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INTRODUCTION

In a recent paper Professor JENNINGS (1916) has given formulae for the calculation of the results of various systems of breeding in which a single Mendelian trait is in question. It seems that JENNINGS's method gave him no absolute assurance of the correctness of his formulae. To quote from his paper (1916, page 62),

"After a law or regular series is obtained that fits the first five or six generations, the law is applied to give the results for three or four generations more. These results are then tested by the actual detailed working out (symbolic formation of gametes and their mating, etc.) for these same later generations; if the formula has given the correct results, it is assumed to be a general formula."

Again (1916, page 61),

"I am compelled, therefore, in most cases, to content myself with giving the actual formulae, leaving their correctness to the test of time."

It is the purpose of this paper, first, to give some examples to show how a method of mathematical repetition can be used to suggest formulae and how mathematical induction can be used to establish a formula when once suggested; second, to express the nth term of series in JENNINGS's table 1, (1916, page 54) as a function of n; third, to solve the problem of inbreeding by brother and sister mating. This paper deals only with a single pair of typical Mendelian factors.

PART I. APPLICATIONS OF THE METHODS OF MATHEMATICAL INDUCTION AND REPETITION

1. *Random mating in a general population*

Consider the problem of random mating in a population consisting of $r\,AA + s\,Aa + t\,aa$. The fundamental method of considering all possible crosses gives the results stated by JENNINGS (1916, page 65) for the first generation:

1) $(s + 2r)^2 AA + 2(s + 2r)(s + 2t)Aa + (s + 2t)^2 aa.$

It should be stated once for all that it is only the relative magnitudes of the coefficients of AA, Aa and aa which are of interest. It has been shown[1] that 1) gives the result for all following generations. A proof will be given here to illustrate a method which is quite valuable for other problems in breeding.

To get the composition of the second generation, one should note that he has merely a repetition of the problem of getting the composition of the first generation. We have to consider the problem of random mating in a population consisting of $R\,AA + S\,Aa + T\,aa$, in which

2) $R = (s + 2r)^2, S = 2(s + 2r)(s + 2t), T = (s + 2t)^2.$

It is needless to repeat the work involved in obtaining expression 1). We read from 1) immediately that the second generation will have the composition

3) $(S + 2R)^2\,AA + 2(S + 2R)(S + 2T)Aa + (S + 2T)^2 aa.$

To find what this means in terms of r, s, t, we substitute the values of R, S, T from 2) into the expression 3). This gives the composition

$$AA = 16(r + s + t)^2(s + 2r)^2$$
$$Aa = 16(r + s + t)^2 2(s + 2r)(s + 2t)$$
$$aa = 16(r + s + t)^2(s + 2t)^2.$$

For want of a better name this process is called "mathematical repeti-

[1] This has been proved by WENTWORTH and REMICK (1916) who state that JENNINGS also had the result.

tion." Omitting the common factor, $16(r + s + t)^2$, which has nothing to do with the proportions involved, we have the same composition for the second generation that we had for the first.

We can read from this result more than a conclusion regarding the second generation. We can say that random mating in any population of composition 1) results in another generation of the same composition. Thus for our original problem, we have the conclusion that after the first random mating the proportions in the population are fixed and are given by expression 1).

2. *A special case of assortative mating*

This example is to illustrate how mathematical induction can be used to test the accuracy of a formula when once suggested. Consider the problem of assortative mating, dominants with dominants, recessives with recessives. Beginning with a cross between AA and aa, and following this by assortative mating for n generations, JENNINGS (1916, page 66) gives the resultant composition as follows:

4) $(n + 1)AA + 2Aa + (n + 1)aa.$

If this composition is correct for a particular value of n, and assortative mating occurs in the population it represents, the next generation should show a composition obtained from 4) by replacing n with $n + 1$. Conversely, if assortative mating in the population 4) gives a population of composition obtained by replacing n by $n + 1$ in 4), and if our original problem gives the distribution 4) for $n = 1$, then the formula 4) holds for all values of n. The most elementary methods show that 4) holds for $n = 1$. Then to complete the proof it is only necessary to show that assortative mating in a population 4) results in a population of composition obtained by replacing n by $n + 1$ in 4); i.e.,

5) $(n + 2)AA + 2Aa + (n + 2)aa.$

In assortative mating the AA and Aa individuals mate at random while the aa individuals mate with like kind. Out of every $2n + 4$ children, $n + 3$ will come from dominant parents, the remaining $n + 1$ coming from recessive parents. The crosses among the dominants will be in the proportions

$$(n + 1)^2 AA \times AA, 4(n + 1)AA \times Aa, 4\, Aa \times Aa.$$

We shall use the notation (a, b, c) to indicate a individuals of type AA, b of type Aa and c of type aa. Then the three crosses noted will produce individuals in the following proportions:

$$\begin{array}{lll} & (\ a, & b, \quad c) \\ (n+1)^2 AA \times AA = & ((n+1)^2, & 0, \quad 0). \\ 4(n+1)AA \times Aa = & (2(n+1), & 2(n+1), \quad 0). \\ 4 \quad Aa \times Aa = & (\ 1, & 2, \quad 1). \\ \text{Totals} & = ((n+2)^2, & 2(n+2), \quad 1). \end{array}$$

Then the $(n+1)$th generation consists of individuals in the following proportions:

$$AA = \frac{(n+2)^2}{(n+3)^2} \cdot \frac{n+3}{2n+4}; \; Aa = \frac{2(n+2)}{(n+3)^2} \cdot \frac{n+3}{2n+4};$$

$$aa = \frac{1}{(n+3)^2} \cdot \frac{n+3}{2n+4} + \frac{n+1}{2n+4} = \frac{(n+2)^2}{(n+3)(2n+4)}$$

Removing the common factor $1/[2(n+3)]$ we have

$$(n+2)AA + 2Aa + (n+2)aa,$$

which is identical with expression 5) as was desired.

3. *Assortative mating in a general population*

As a final example illustrating both methods, consider the more general problem of assortative mating of the population

$$r\,AA + s\,Aa + t\,aa.$$

Detailed examination of the crosses involved gives the result stated by JENNINGS (1916, page 67) for the first generation,

6) $(2r+s)^2 AA + 2s(2r+s)Aa + (s^2 + 4rt + 4st)aa.$

The problem is now really simpler than was the special case considered above. To get the composition of the second generation we need not consider the crosses involved at all. If we set

7) $(2r+s)^2 = R,\ 2s(2r+s) = S,\ s^2 + 4rt + 4st = T,$

expression 6) can be written

$$R\,AA + S\,Aa + T\,aa.$$

We seek the result of assortative mating in this population and it is evident that it is only necessary to write expression 6) with large letters. The second generation has the composition,

8) $(2R+S)^2 AA + 2S(2R+S)Aa + (S^2 + 4RT + 4ST)aa.$

To interpret this we must replace R, S, T by their values in r, s, t from equations 7).

$$(2R+S)^2 = 4(2r+s)^2(2r+2s)^2.$$
$$2S(2R+S) = 4(2r+s)^2 2s(2r+2s).$$
$$S^2 + 4RT + 4ST = 4(2r+s)(2r+2s)(2s^2 + 4rt + 6st).$$

Omitting the common factor $4(2r + s)(2r + 2s)$, we have for the second generation

9) $(2r + s)(2r + 2s)AA + 2s(2r + s)Aa + (2s^2 + 4rt + 6st)aa.$

This, or at least one more repetition of the process, suggests that the nth generation will have the composition[2]

10) $(2r + s)(2r + ns)AA + 2s(2r + s)Aa + [ns^2 + 4rt + 2(n + 1)st]aa.$

Inspection shows that this formula holds for $n = 1$ and $n = 2$. If we assume 10) thinking of n as fixed, and show that assortative mating in such a population gives a generation whose composition is obtained by replacing n by $n + 1$ in 10), then we shall know that 10) holds for all values of n. To do this let

$R = (2r + s)(2r + ns)$; $S = 2s(2r + s)$; $T = ns^2 + 4rt + 2(n + 1)st$,

and form expression 6) in the large letters; i.e., the expression 8) with our present meaning for R, S, T. This process gives for the proportions in the $(n + 1)$th generation.

$$AA = 4(2r + s)^2[2r + (n + 1)s]^2.$$
$$Aa = 4(2r + s)^2 . 2s[2r + (n + 1)s].$$
$$aa = 4(2r + s)[2r + (n + 1)s][n + 1)s^2 + 4rt + 2(n + 2)st].$$

Dividing by the common factor $4(2r + s)[2r + (n + 1)s]$ the proportions become,

$$(2r + s)[2r + (n + 1)s]AA + 2s(2r + s)Aa + [(n + 1)s^2 + 4rt + 2(n + 2)st]aa.$$

Inspection shows that these results may be obtained by replacing n by $n + 1$ in expression 10).

It should be of interest to note that as n increases indefinitely the proportions in 10) approach the proportions in

$$(2r + s)AA + 0\,Aa + (2t + s)aa.$$

These examples should show, first that the method of mathematical repetition can be used to simplify the work of calculating the composition of higher generations; second, that the method of mathematical induction can be used to prove or disprove a general formula for the composition of the nth generation when it has once been suggested.

PART II. GENERAL TERMS OF JENNINGS'S SERIES

In table 1 JENNINGS (1916, page 54) gives twenty terms of each of several series which present themselves in breeding problems. For series

[2] This result was obtained by WENTWORTH and REMICK (1916).

B, C; D and E he gives the nth term as a function of n. It may be desirable to have the nth term of his other series (lettered from F to M). Inspection shows that only two of these are independent and if we can express the nth term of each of them, the others come immediately. The derivation of these two nth terms will be given next and then the nth term of each series will be written down.

1. *Derivation of the nth term of the Fibonacci series*

The Fibonacci series F is defined by its first two terms, $F_0 = 0$, $F_1 = 1$, and the recurrence relation $F_n = F_{n-1} + F_{n-2}$. In mathematical language we have to solve the homogeneous recurrence equation

11) $F_n - F_{n-1} - F_{n-2} = 0$

with the initial conditions, $F_0 = 0$ and $F_1 = 1$. It is well known that C^n is a solution of 11), where C is a root of $C^2 - C - 1 = 0$; i.e., $C = (1 \pm \sqrt{5})/2$. Then $[(1 + \sqrt{5})/2]^n$ and $[(1 - \sqrt{5})/2]^n$ are solutions of 11) and any solution can be put in the form

12) $F_n = [K_1(1 + \sqrt{5})^n + K_2(1 - \sqrt{5})^n]/2^n$.

We wish to determine the constants K_1 and K_2 so that $F_0 = 0$ and $F_1 = 1$. Setting $n = 0$ and $n = 1$ in equation 12), we have

13) $F_0 = K_1 + K_2 = 0$.

14) $F_1 = [K_1(1 + \sqrt{5}) + K_2(1 - \sqrt{5})]/2 = 1$.

From 13), $K_1 = -K_2$. Substituting in 14),

$$F_1 = K_1[1 + \sqrt{5} - (1 - \sqrt{5})]/2 = 1.$$

$K_1 = 1/\sqrt{5}$; $K_2 = -1/\sqrt{5}$; and

15) $F_n = [(1 + \sqrt{5})^n - (1 - \sqrt{5})^n]/[\sqrt{5} \cdot 2^n]$.

The rather complicated appearance of this formula may make it seem useless. If one desires only a few of the early terms in the series, it would most certainly not be advisable to use this formula. But suppose you want the 100th term. By using logarithms it is about as easy to get the 100th term with all desirable accuracy from this formula 15) as it is to get the tenth term, and no time need be spent calculating the first 99 terms.

The formula 15) for the Fibonacci series enables us to prove the following important

THEOREM: *As n increases indefinitely, the nth term of the Fibonacci series divided by 2^n approaches zero as a limit.*

Symbolically stated, the theorem is

16) $\lim_{n=\infty} F_n/2^n = 0$.

Writing in the value of F_n this becomes

$$\underset{n=\infty}{\text{Limit}} \frac{(1+\sqrt{5})^n-(1-\sqrt{5})^n}{\sqrt{5}\cdot 4^n}=0.$$

The proof consists in noting that $(1+\sqrt{5})/4$ and $(1-\sqrt{5})/4$ are proper fractions and that as a proper fraction is raised to higher and higher powers, the result approaches zero as a limit. As an immediate corollary we have that if C_1 and c_2 are constants,

$$\underset{n=\infty}{\text{Limit}} \; C_1F_n/2^{n+c_2}=0.$$

This follows because $C_1/2^{c_2}$ is a constant, say C_3, and we have

$$\underset{n=\infty}{\text{Limit}} \frac{C_3F_n}{2^n}=C_3\underset{n=\infty}{\text{Limit}}\frac{F_n}{2^n}=0.$$

2. *Derivation of series* G

The second series which it is necessary to consider is defined by the recurrence $G_n=2^{n-1}-G_{n-1}$, together with the initial condition $G_0=0$. We have to solve the non-homogeneous recurrence

17) $G_n+G_{n-1}=2^{n-1}$

subject to the condition $G_0=0$. The most general solution is the sum of the general solution of the homogeneous equation

18) $G_n+G_{n-1}=0$

and any particular solution of equation 17). The general solution of 18) is KC^n where C is a solution of $C+1=0$; i.e., $K(-1)^n$. A particular solution of equation 17) is $G_n=2^n/3$.

The general solution of 17) is therefore

19) $G_n=K(-1)^n+2^n/3$.

We wish to determine K so that $G_0=0$. Setting $n=0$ in 19), we have

$G_0=K+1/3 \therefore K=-1/3$ and

20) $G_n=[2^n-(-1)^n]/3$.

The value of a formula for G_n is particularly apparent in an example given by JENNINGS (1916, page 80). The series $G_n.G_{n+1}/2^{2n-1}$ is needed. Substituting the value of G_n and G_{n+1} this fraction is

$$\frac{1}{9}\left[\frac{2^{2n+1}-(-2^n)-1}{2^{2n-1}}\right]=\frac{4}{9}-\frac{(-2)^n+1}{9\cdot 2^{2n-1}}.$$

From this expression, the various terms of the series can be calculated readily, independently, and without recourse to any complicated rule, and the limit approached as n increases indefinitely is apparent.

3. *The nth terms of series in* JENNINGS's *table*

Using the values of F_n and G_n we can write down the following set of nth terms for JENNINGS's series:

$$F_n = \frac{(1+\sqrt{5})^n - (1-\sqrt{5})^n}{\sqrt{5} \cdot 2^n}; \quad G_n = \frac{1}{3}\left[2^n - (-1)^n\right]; \quad B_n = 2^n.$$

$$H_n = G_n - F_n = \frac{1}{3}\left[2^n - (-1)^n\right] + \frac{(1-\sqrt{5})^n - (1+\sqrt{5})^n}{\sqrt{5} \cdot 2^n}.$$

$$I_n = B_n - G_n - F_n = \frac{2^{n+1} + (-1)^n}{3} + \frac{(1-\sqrt{5})^n - (1+\sqrt{5})^n}{\sqrt{5} \cdot 2^n}.$$

$$J_n = B_n - F_{n+1} = 2^n + \frac{(1-\sqrt{5})^{n+1} - (1+\sqrt{5})^{n+1}}{\sqrt{5} \cdot 2^{n+1}}.$$

$$K_n = B_n - F_{n+2} = 2^n + \frac{(1-\sqrt{5})^{n+2} - (1+\sqrt{5})^{n+2}}{\sqrt{5} \cdot 2^{n+2}}.$$

$$L^3_n = B_n - G_{n-1} - F_{n-1} = \frac{5 \cdot 2^{n-1} + (-1)^{n-1}}{3} + \frac{(1-\sqrt{5})^{n-1} - (1+\sqrt{5})^{n-1}}{\sqrt{5} \cdot 2^{n-1}}.$$

$$M_n = 3\ B_n - F_{n+2} = 3\ \ 2^n + \frac{(1-\sqrt{5})^{n+2} - (+\sqrt{5})^{n+2}}{\sqrt{5} \cdot 2^{n+2}}.$$

Incidentally it may be noted that E_n, given by JENNINGS as $2^{n-1} + 2^{n-2} - 1$ can be written in the slightly more compact form, $E_n = 3 \cdot 2^{n-2} - 1$.

PART III. BROTHER AND SISTER MATING

1. *Results in random brother and sister mating*

Given a family consisting of $r\,AA + s\,Aa + t\,aa$, what is the composition of the nth generation if mating is restricted to random mating between brothers and sisters? Special cases of this problem have been considered by JENNINGS (1916) and PEARL (1914).

For the benefit of those who do not care to follow the details of the development, the results will be stated first. The nth generation, i.e., the generation resulting from the nth brother and sister mating, has the following composition:

$AA = [1 + K_2 - T_n]/2$; $Aa = T_n$; $aa = [1 - K_2 - T_n]/2$, in which $T_n = [s\,K\,F_{n+1} + (rs + st + 4rt)F_n]/K^2 \cdot 2^n$; $K_2 = (r-t)/K$; $K = r + s + t$; and F_n is the general term of the Fibonacci series.

[3] By what is evidently a slip, JENNINGS writes F_{n+1} in this equation for F_{n-1}.

2. *Development of above results*

Three types of individuals are involved, *AA*, *Aa* and *aa*. The different possible crosses of individuals of these types together with the composition of the resulting families are given below. The notation (a, b, c) means that individuals of the types *AA*, *Aa*, *aa* appear in numbers proportional to a, b, c.

Kind of cross	Composition of resulting family	Letter indicating type of family
$AA \times AA$	(1, 0, 0)	o
$AA \times Aa$	(½, ½, 0)	p
$AA \times aa$	(0, 1, 0)	q
$Aa \times Aa$	(¼, ½, ¼)	r
$Aa \times aa$	(0, ½, ½)	u
$aa \times aa$	(0, 0, 1)	v

It is useful to keep track of these six kinds of families. Let o_n, p_n, q_n, r_n, u_n, v_n be the relative numbers of families of the various kinds in the order given above. If we can calculate $o_n \ldots\ldots v_n$ we can readily find the numbers of *AA*, *Aa*, *aa* individuals in the nth generation.

a. *Development of the formulae for* $o_n, p_n, q_n, r_n, u_n, v_n$

To find o_n, for instance, we examine the source of the families in the nth generation of the type o. All the children of families of type o in the $(n-1)$th generation will be in families of type o, since *AA* individuals only are concerned. One-fourth of the families which consist of children of families of type p in the $(n-1)$th generation will be of type o and 1/16 of the families which are children of families of type r in the $(n-1)$th generation will be of type o. Thus we have that[4]

21) $o_n = o_{n-1} + p_{n-1}/4 + r_{n-1}/16.$

Similar considerations give

22) $p_n = p_{n-1}/2 + r_{n-1}/4.$

23) $q_n = r_{n-1}/8.$

24) $r_n = p_{n-1}/4 + q_{n-1} + r_{n-1}/4 + u_{n-1}/4.$

25) $u_n = u_{n-1}/2 + r_{n-1}/4.$

26) $v_n = v_{n-1} + u_{n-1}/4 + r_{n-1}/16.$

The problem before us is to solve this system of recurrence relations.

[4] PEARL (1914) had these equations, except that in the case he considered, $o_n = v_n$; $p_n = u_n$. The notation here used was used by PEARL.

We first set

27) $p_n - u_n = y_n$, and

28) $o_n - v_n = x_n$.

Then from equations 22) and 25),

29) $y_n = y_{n-1}/2$, and similarly

30) $x_n = x_{n-1} + y_{n-1}/4$ and the above system, 21)-26), may be replaced by the following system:

21′) $o_n = o_{n-1} + p_{n-1}/4 + r_{n-1}/16$.

22′) $p_n = p_{n-1}/2 + r_{n-1}/4$.

23′) $r_n = r_{n-1}/4 + r_{n-2}/8 + p_{n-1}/2 - y_{n-1}/4$.

24′) $y_n = y_{n-1}/2$.

25′) $x_n = x_{n-1} + y_{n-1}/4$.

Equation 24′) may be written

$$2y_n - y_{n-1} = 0.$$

The most general solution of this equation is

31) $y_n = K_1/2^n$, in which K_1 is an arbitrary constant. Then equation 25′) becomes

$$x_n - x_{n-1} = K_1/2^{n+1}.$$

The most general solution of this equation is

32) $x_n = K_2 - K_1/2^{n+1}$, K_2 being an arbitrary constant.

From equation 22′)

33)
$$\begin{cases} r_{n-1} = 4p_n - 2p_{n-1}, \\ r_{n-2} = 4p_{n-1} - 2p_{n-2}, \\ r_n = 4p_{n+1} - 2p_n. \end{cases}$$

Substituting these values of r_n, r_{n-1}, r_{n-2}, in equation 23′) and using equation 31) gives the equation

34) $16p_{n+1} - 12p_n - 2p_{n-1} + p_{n-2} = -K_1/2^{n-1}$.

The corresponding algebraic equation is $16c^3 - 12c^2 - 2c + 1 = 0$; the roots are $c = 1/4$; $c = (1 + \sqrt{5})/4$; $c = (1 - \sqrt{5})/4$. Then the most general solution of the homogeneous equation

$$16p_{n+1} - 12p_n - 2p_{n-1} + p_{n-2} = 0 \text{ is}$$

$$[K_3(1 + \sqrt{5})^n + K_4(1 - \sqrt{5})^n + K_5]/4^n,$$

in which K_3, K_4 and K_5 are arbitrary constants. A particular solution of the non-homogeneous equation 34) is $K_1/2^{n+1}$. Therefore the general solution of equation 34) is

35) $$p_n = \frac{K_1}{2^{n+1}} + \frac{K_3(1 + \sqrt{5})^n + K_4(1 - \sqrt{5})^n + K_5}{4^n}$$

Let $P_n = K_3(1 + \sqrt{5})^n + K_4(1 - \sqrt{5})^n$.

Then 35) may be written,

36) $p_n = K_1/2^{n+1} + (P_n + K_5)/4^n$.

From $y_n = p_n - u_n$, we have $u_n = p_n - y_n = p_n - K_1/2^n$.

37) $u_n = -K_1/2^{n+1} + (P_n + K_5)/4^n$.

From 33) $r_n = 4p_{n+1} - 2p_n$. A little algebraic reduction shows that this becomes

38) $r_n = [4P_{n-1} - K_5]/4^n$.

Since $q_n = r_{n-1}/8$, we have

39) $q_n = (4P_{n-2} - K_5)/2 \times 4^n$.

By direct substitution one can verify that

$$P_n - 2P_{n-1} - 4P_{n-2} = 0.$$

Using this equation, q_n may be written

40) $q_n = [P_n - 2P_{n-1} - K_5]/2 \times 4^n$.

Finally, to get o_n and v_n we note that since o_n ..... v_n are only proportional to the numbers of families of different types, it will simplify the problem to choose them so that,

$$o_n + p_n + q_n + r_n + u_n + v_n = 1.$$

Then $o_n + v_n = 1 - (p_n + q_n + r_n + u_n)$.

From equation 32) we have that

$$o_n - v_n = K_2 - K_1/2^{n+1}.$$

Solving the last two equations for o_n and v_n,

41) $o_n = -K_1/2^{n+2} + \frac{K_2 + 1}{2} - \frac{1}{2}(p_n + q_n + r_n + u_n)$.

42) $v_n = K_1/2^{n+2} - \frac{K_2 - 1}{2} - \frac{1}{2}(p_n + q_n + r_n + u_n)$.

Substituting the values of p_n, q_n, r_n, u_n, from equations 36), 37), 38), 40) into equations 41), 42) gives,

43) $o_n = \frac{1 + K_2}{2} - \frac{K_1}{2^{n+2}} - \frac{5P_n + 6P_{n-1} + K_5}{4^{n+1}}$.

44) $v_n = \frac{1 - K_2}{2} + \frac{K_1}{2^{n+2}} - \frac{5P_n + 6P_{n-1} + K_5}{4^{n+1}}$.

The constants $K_1 \ldots . K_5$ are to be found in terms of the initial conditions; in our problem they are functions of r, s, t. To determine them we need the values of o_1, p_1, q_1, r_1, u_1, v_1. Considering the possible crosses involved in mating the family $r\,AA + s\,Aa + t\,aa$ and using the notation $K = r + s + t$, we find that

$$o_1 = \frac{r^2}{K^2}; \; p_1 = \frac{2rs}{K^2}; \; q_1 = \frac{2rt}{K^2};$$

$$r_1 = \frac{s^2}{K^2}; \; u_1 = \frac{2st}{K^2}; \; v_1 = \frac{t^2}{K^2}.$$

To evaluate K_1 we note from equation 31) that $y_1 = K_1/2$. Also $y_1 = p_1 - u_1$ by definition. Then $K_1 = 2y_1 = 2(p_1 - u_1)$ and substituting for p_1, u_1,

$$45) \quad K_1 = \frac{4s(r-t)}{K^2}.$$

From equation 32), $K_2 = x_1 + K_1/4 = o_1 - v_1 + K_1/4$; and substituting for o_1, v_1,

$$46) \quad K_2 = \frac{r-t}{K}.$$

More complicated work of the same nature gives for the remaining constants,

$$47) \quad K_3 = \frac{(1+\sqrt{5})s}{5K} + \frac{(1-\sqrt{5})}{5K^2}(s^2 - 4rt).$$

$$48) \quad K_4 = \frac{(1-\sqrt{5})s}{5K} + \frac{(1+\sqrt{5})}{5K^2}(s^2 - 4rt).$$

$$49) \quad K_5 = \frac{4}{5K^2}[s(2r - s + 2t) - 8rt].$$

It should be noted that we have here five constants $K_1 \ldots K_5$ expressed in terms of three initial numbers r, s, t. This indicates that our method is useful for a more general problem than the one to which it is here applied. This is shown clearly by expressing $K_1 \ldots K_5$ in terms of $o_1, p_1 \ldots v_1$ as follows:

$$K_1 = 2(p_1 - u_1); \; K_2 = o_1 - v_1 + (p_1 - u_1)/2.$$

$$K_3 = [(1+\sqrt{5})(p_1 + u_1) + 4(\sqrt{5} - 1)q_1 + 4r_1]/10.$$

$$K_4 = [(1-\sqrt{5})(p_1 + u_1) - 4(\sqrt{5} + 1)q_1 + 4r_1]/10.$$

$$K_5 = 4[p_1 + u_1 - 4q_1 - r_1]/5.$$

With this set of values of $K_1 \ldots K_5$ our formulae will give the composition of the population after $n - 1$ brother and sister matings starting with families of the six special types in numbers proportional to $o_1, p_1, q_1, r_1, u_1, v_1$.

b. Proportions of the three types of individuals in the nth generation

The final results desired are the numbers giving the proportions of

AA, Aa, aa individuals in the *n*th generation. It is readily seen that they are[5]

$$AA = o_n + p_n/2 + r_n/4.$$
$$Aa = (p_n + r_n + 2q_n + u_n)/2.$$
$$aa = r_n/4 + u_n/2 + v_n.$$

Substituting the values of $o_n \ldots . v_n$,

$$50) \quad AA = \frac{1 + K_2}{2} - \frac{3P_n + 2P_{n-1}}{4^{n+1}}.$$

$$51) \quad Aa = \frac{3P_n + 2P_{n-1}}{2 \times 4^n}.$$

$$52) \quad aa = \frac{1 - K_2}{2} - \frac{3P_n + 2P_{n-1}}{4^{n+1}}.$$

The expression $3P_n + 2P_{n-1}$ which enters these three equations is

$$3P_n + 2P_{n-1} = \frac{\sqrt{5}}{2}[K_3(1 + \sqrt{5})^{n+1} - K_4(1 - \sqrt{5})^{n+1}].$$

It is instructive to get the proportions in 50), 51), 52) in another form by substituting the values of K_3 and K_4 from equations 47) and 48). This gives

$$Aa = \frac{1}{2^n}\left[\frac{s\,F_{n+2}}{K} - \frac{s^2 - 4rt}{K^2}\,F_n\right].$$

in which F_n is the *n*th term of the Fibonacci series. Since $F_{n+2} = F_{n+1} + F_n$,

$$53) \quad Aa = [sK\,F_{n+1} + (rs + st + 4rt)F_n] \div [2^n \cdot K^2].$$

in which $K = r + s + t$.

From this form we can read the following results:

1. *If the numbers representing the proportions of Aa individuals in successive generations be written with* 2^n *in the denominators, the numerators will satisfy the recurrence,*

$$N_n = N_{n-1} + N_{n-2}.$$

2. *If* $s = 0$ *or* $s^2 = 4rt$, *and the denominators are chosen as* $2^nK^2/(s^2 - 4rt)$ *or* $2^nK/s$, *the numerators will be terms of the Fibonacci series.*

3. *As the number of generations increases, the proportion of heterozygous individuals approaches zero regardless of the values of r, s, t.*

4. *As the number of generations increases, the ratio of AA to aa indi-*

[5] PEARL (1914) had this result for *AA* but seems to have erred in getting the numbers for *Aa*. In the case he considered, $o_n = v_n$ and $p_n = u_n$.

viduals approaches $(2r + s)/(2t + s)$, *which is the same as the ratio of A and a gametes in the original family.*

c. Illustrative example

As a check on these formulae, and to illustrate their application, let us take a special case considered by JENNINGS (1916). Let AA and aa be crossed and assume brother and sister mating thereafter. The children of the original cross are all of type Aa. It is with crosses of these individuals that our problem begins. We therefore have $r = t = 0; s = 1$. Substituting in equations 45) — 49),

$K_1 = K_2 = 0; K_3 = K_4 = 2/5; K_5 = -4/5.$

Substituting these values of the constants into equations 50), 51), 52), and using the notation of part II, $F_n = [(1 + \sqrt{5})^n - (1 - \sqrt{5})^n] / \sqrt{5} \cdot 2^n$,

$$AA = \tfrac{1}{2} - F_{n+1}/2^{n+1}; \quad Aa = F_{n+1}/2^n;$$
$$aa = \tfrac{1}{2} - F_{n+1}/2^{n+1}.$$

These results agree with JENNINGS's series.

3. *Assortative brother and sister mating*

Given a family consisting of $r\,AA + s\,Aa + t\,aa$, what is the composition of the nth generation if mating is restricted (1) to brothers with sisters and (2) to dominants with dominants and recessives with recessives?

To derive the recurrence relations upon which the solution of this problem depends we note:

a) Families of type q will not appear since they arise only by a cross between AA and aa.

b) Families of type u will not appear since they arise only by a cross between Aa and aa.

c) Random mating will occur in families of types o, p, v.

d) Assortative mating will occur in families of type r, ¾ of the resulting families being of type o, p, r, in the proportion 1 : 4 : 4 and ¼ being of the type v.

These considerations lead to the following equations:

$$54) \quad o_n = o_{n-1} + \frac{p_{n-1}}{4} + \frac{r_{n-1}}{12}.$$

$$55) \quad p_n = \frac{p_{n-1}}{2} + \frac{r_{n-1}}{3}.$$

56) $r_n = \frac{p_{n-1}}{4} + \frac{r_{n-1}}{3}$.

57) $v_n = v_{n-1} + \frac{r_{n-1}}{4}$.

The problem of solving this system of equations is very similar to the problem considered above in studying random brother and sister mating. Using the notation

$$P_n = K_1(5 + \sqrt{13})^n + K_2(5 - \sqrt{13})^n,$$

the solution takes the form,

58) $o_n = 1 - K_3 - 3P_{n+1}/2 \times 12^{n+1}$.

59) $p_n = P_n/12^n$.

60) $r_n = (P_{n+1} - 6P_n)/4 \times 12^n$.

61) $v_n = K_3 - (P_{n+1} - 4P_n)/8 \times 12^n$.

The proportions of the three types of individuals in the nth generation are given by

62) $AA = o_n + \frac{p_n}{2} + \frac{r_n}{4} = 1 - K_3 - \frac{P_{n+1} - 2P_n}{16 \cdot 12^n}$.

63) $Aa = \frac{p_n + r_n}{2} = \frac{P_{n+1} - 2P_n}{8 \cdot 12^n}$.

64) $aa = v_n + \frac{r_n}{4} = K_3 - \frac{P_{n+1} - 2P_n}{16 \cdot 12^n}$.

We have to determine the constants K_1, K_2, K_3 in terms of the initial numbers r, s, t. First, substituting $n = 1$ in equations 58), 59), 60). 61), and solving,

65) $K_1 = 2[(\sqrt{13} - 2)p_1 + (5 - \sqrt{13})r_1]/\sqrt{13}$.

66) $K_2 = 2[(\sqrt{13} + 2)p_1 - (5 + \sqrt{13})r_1]/\sqrt{13}$.

67) $K_3 = [2(1 + v_1 - o_1) - p_1]/4$.

Examination of the first matings shows that

68) $o_1 = \frac{r^2}{(r+s)K}$; $p_1 = \frac{2rs}{(r+s)K}$; $r_1 = \frac{s^2}{(r+s)K}$; $v_1 = \frac{t}{K}$,

in which $K = r + s + t$.

Substituting these values into equations 65), 66), 67), we have,

69) $K_1 = 2s[2r(\sqrt{13} - 2) + s(5 - \sqrt{13})]/[\sqrt{13} \cdot K(r + s)]$.

70) $K_2 = 2s[2r(\sqrt{13} + 2) - s(\sqrt{13} + 5)]/[\sqrt{13} \cdot K(r + s)]$.

71) $K_3 = (2t + s)/2K$.

The expressions for AA, Aa, aa, in terms of r, s, t, are far from neat.

The one for Aa will be given; those for AA and aa can be readily calculated from the one for Aa by using equations 62), 63), 64).

$$72) \quad Aa = \frac{s}{2K\sqrt{13}\cdot 12^n (r+s)}\left[(5+\sqrt{13})^n\{(7+\sqrt{13})r+(1+\sqrt{13})s\}+ (5-\sqrt{13})^n\{(-7+\sqrt{13})r-(1-\sqrt{13})s\}\right].$$

It is instructive to note that

$$\underset{n=}{\text{Limit}} \frac{P_n}{12^{n+c}} = 0.$$

This follows from the fact that $(5+\sqrt{13})/12$ and $(5-\sqrt{13})/12$ are proper fractions, and that a proper fraction raised to higher and higher powers approaches zero as a limit. With this in mind we see at once from equation 63) that the proportion of heterozygotes approaches zero as n increases. Then

$$\underset{n=\infty}{\text{Limit}} (AA)_n = 1 - K_3 = \frac{2r+s}{2K}, \text{ and}$$

$$\underset{n=\infty}{\text{Limit}} (aa)_n = K_3 = \frac{2t+s}{2K}.$$

Here again we see what has been true of every problem in inbreeding, that the heterozygotes tend to disappear and the homozygotes approach the proportion

$$AA/aa = (2r+s)/(2t+s).$$

This is to be expected. In fact the following statement of the case seems obvious:

Any method of breeding which gives A and a gametes equal chances of mating and which tends to eliminate heterozygous individuals will in successive generations give populations which approach a stable condition in which the two types of homozygous individuals appear in the same proportion as were their types of gametes in the original population.

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